

PUB-NO: WO009206197A1
DOCUMENT-IDENTIFIER: WO 9206197 A1
TITLE: METHOD OF TESTING FOR SALMONELLA

PUBN-DATE: April 16, 1992

INVENTOR-INFORMATION:

NAME	COUNTRY
THORNS, CHRISTOPHER JOHN	GB

INT-CL (IPC): C12N 1/20; C12N 15/31; C12N 15/62; C12P 21/08; G01N 33/569
EUR-CL (EPC): C07K014/35; C07K016/12, C12Q001/10 , G01N033/569

ABSTRACT:

CHG DATE=19990617 STATUS=O>A method of testing for the presence of Salmonella serotypes S. enteritidis and S. dublin is provided. Novel monoclonal antibodies are used to detect the presence of an epitope specific for these serotypes in cultures which have been grown on selected media which enhance the expression of said epitope in fimbrial sites. Test kits utilising the antigen or its epitopic parts, antibodies and/or the media are further provided.

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PUB-NO: WO009425597A2

DOCUMENT-IDENTIFIER: WO 9425597 A2

TITLE: METHODS AND COMPOSITIONS FOR DETECTION OF SALMONELLA

PUBN-DATE: November 10, 1994

INVENTOR-INFORMATION:

NAME	COUNTRY
DORAN, JAMES L	CA
KAY, WILLIAM W	CA
COLLINSON, S KAREN	CA
CLOUTHIER, SHARON C	CA

INT-CL (IPC): C12N 15/31; C12Q 1/68; C12P 21/08; C07K 14/255; G01N 33/56; C12N 5/12

EUR-CL (EPC): C07K014/255; C12N015/52, C12Q001/68 , C07K016/12

ABSTRACT:

Isolated nucleic acid molecules comprising one or more of the sefU?1?, sefU?2?, sefA, sefB, sefC, sefD, agfA, tctA, tctB, or tctC genes of Salmonella and a gene cluster that contains one or more of such genes, such as the sefU?2?U?1? gene cluster, the sefBCD gene cluster, and the tctI, tctII or tctIII gene clusters. Probes and primers complementary to or derived from said genes. Isolated proteins encoded by said genes. Methods and composition suitable for diagnostic tests utilizing the isolated genes and proteins to give highly specific diagnostic assays to Salmonella, S. enteritidis, S. dublin, S. gallinarum, and/or enteropathogenic bacteria of the family Enterobacteriaceae.

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IPCR/8 classification codes now searchable as IC=. See HELP NEWSIPCR.

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Set	Items	Description
S1	1	'SEF 14'
S2	230	E3-E10
S3	1	'SEF-14 FIMBRIAE ANTIGEN'
S4	244	'SEFA' OR 'SEFA ANTIGEN' OR 'SEFA PROTEIN, SALMONELLA ENTE- RITIDIS' OR 'SEFA PROTEIN'
S5	1	'SEF-A'
S6	386	S1 OR S2 OR S3 OR S4 OR S5
S7	227	S6/1999:2006
S8	159	S6 NOT S7
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9/9/1 (Item 1 from file: 155)
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12045245 PMID: 9884830

Application of recombinant fimbrial protein for the specific detection of Salmonella enteritidis infection in poultry.

Rajashekara G; Munir S; Lamichhane C M; Back A; Kapur V; Halvorson D A; Nagaraja K V

Department of Veterinary PathoBiology, University of Minnesota, St. Paul 55108, USA.

Diagnostic microbiology and infectious disease (UNITED STATES) Nov 1998
, 32 (3) p147-57, ISSN 0732-8893--Print Journal Code: 8305899.

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

A number of disease outbreaks of Salmonella enterica serotype enteritidis (SE) in humans have been traced to the consumption of SE-contaminated egg and egg products. A rapid, specific, and inexpensive method of detecting SE infection in poultry is necessary to reduce human outbreaks. We evaluated rSEF14 fimbrial antigen of SE for specific detection of SE-infected birds in latex agglutination test and enzyme-linked immunosorbent assay. rSEF14 antigen was highly specific in identifying birds infected with SE. The sera from birds infected with closely related serogroup-D Salmonella and other avian pathogens did not react with rSEF14 antigen. The rSEF14 antigen identified antibodies in serum of 88% of birds during the first 2 weeks of infection, and 100% of the birds subsequently. The SE-specific antibodies were detected in egg yolk as early as 6 days post-infection in rSEF14-enzyme-linked immunosorbent assay. Our results suggest that rSEF14-based assays could be used as screening tests for detection of SE antibodies and would overcome the cross reactions observed with existing serological tests.

Descriptors: *Bacterial Proteins--immunology--IM; *Chickens; *Fimbriae

INTERNATIONAL SEARCH REPORT

International Application No

PCI/SG 99/00061

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/10 C07K14/255

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	<p>WO 98 03656 A (RAJASHEKARA GIREESH ;KAPUR VIVEK (US); UNIV MINNESOTA (US); NAGARA) 29 January 1998 (1998-01-29) page 3, line 14 - line 22</p> <p>page 5, line 20 - line 25 page 17, line 4 - line 30; claims 1,4,10 --- -/--</p>	<p>1-3,5, 16,17,23</p> <p>6,14,15, 18,23</p>



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

22 May 2000

Date of mailing of the international search report

13. 06. 2000

Name and mailing address of the ISA

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Authorized officer

Gundlach, B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/SG 99/00061

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>ASTEN VAN A J A M ET AL: "IDENTIFICATION OF THE DOMAIN WHICH DETERMINES THE G,M SEROTYPE OF THE FLAGELLIN OF SALMONELLA ENTERITIDIS"</p> <p>JOURNAL OF BACTERIOLOGY,US,WASHINGTON, DC, vol. 177, no. 6, 1 March 1995 (1995-03-01), pages 1610-1613, XP002069554</p> <p>ISSN: 0021-9193</p> <p>page 1613, column 1, line 14 - line 16; figure 1</p> <p style="text-align: center;">---</p>	1-4, 7-17, 19-34
Y	<p>BARROW P A: "SEROLOGICAL DIAGNOSIS OF SALMONELLA SEROTYPE ENTERITIDIS INFECTIONS IN POULTRY BY ELISA AND OTHER TESTS"</p> <p>INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 21, no. 1/02, 1 January 1994 (1994-01-01), pages 55-68, XP000198428</p> <p>ISSN: 0168-1605</p> <p>cited in the application</p> <p>the whole document</p> <p style="text-align: center;">---</p>	1-4, 7-17, 19-34
A	<p>COOPER G L ET AL: "EVALUATION OF SEF14 FIMBRIAL DOT BLOT AND FLAGELLAR WESTERN BLOT TEST AS INDICATORS OF SALMONELLA ENTERITIDIS INFECTION IN CHICKENS"</p> <p>VETERINARY RECORD,GB,LONDON, vol. 138, no. 7, 17 February 1996 (1996-02-17), pages 149-153, XP000198431</p> <p>ISSN: 0042-4900</p> <p>page 152, column 2, paragraph 3 -page 153, column 1, paragraph 1</p> <p>page 149, column 2, paragraph 3</p> <p style="text-align: center;">---</p>	1-34
A	<p>GAST, R.K. ET AL.: "Assessing the Sensitivity of Egg Yolk Antibody Testing for Detecting Salmonella Enteritidis Infections in Laying Hens."</p> <p>POULTRY SCIENCES, vol. 76, 1997, pages 798-801, XP000909155</p> <p>the whole document</p> <p style="text-align: center;">---</p>	1-4, 7-17, 19-34
A	<p>GAST, R.K. ET AL.: "Applying Tests for Specific Yolk Antibodies to Predict Contamination by Salmonella enteritidis in Eggs from Experimentally Infected Laying Hens"</p> <p>AVIAN DISEASES, vol. 41, 1997, pages 195-202, XP000910421</p> <p>the whole document</p> <p style="text-align: center;">---</p>	1-4, 7-17, 19-34
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NCBI BLAST program reference [PMID:9254694]:
Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402(1997).

=====

Query: 165 AA (of which 26% low-complexity regions filtered out)
Date run: 2006-06-05 11:33:09 UTC+0100 on blast01.vital-it.ch
Program: NCBI BLASTP 2.2.13 [Nov-27-2005]
Database: UniProtKB
3,185,498 sequences; 1,044,150,180 total letters
UniProt Knowledgebase Release 8.0 consists of:
UniProtKB/Swiss-Prot Release 50.0 of 30-May-2006: 222289 entries
UniProtKB/TrEMBL Release 33.0 of 30-May-2006: 2948323 entries

List of potentially matching sequences

Send selected sequences to

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Db	AC	Description
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- | | | |
|--------------------------|-----------|---|
| <input type="checkbox"/> | sp P12061 | FM_SALEN Fimbrial protein precursor [sefA] [Salmonella. |
| <input type="checkbox"/> | tr Q5PM43 | _SALPA Fimbrial structural protein [sefA] [Salmonella p |

tr Q5PM43 Fimbrial structural protein [sefA] [Salmonella
Q5PM43_SALPA paratyphi-a]

Score = 224 bits (572), Expect = 6e-58
Identities = 116/165 (70%), Positives = 118/165 (71%)

Query: 1 MRKXXXXXXXXXXIACGSAHAAGFVGNKXXXXXXXXXXXXXXXXXSANWSQDPGFTGP
MRK IACGSA+AAGFVGNK SANWSQDPGFTGP
Sbjct: 1 MRKSASAVAVLALIACGSAYAAGFVGNKAEVQAAVTIAAQNTTSANWSQDPGFTGP

Query: 61 GQKVGTLSTATGPHNXXXXXXXXXXXXXXXXXXXXPFVDGQGQPVFRGRIQGANIND
GQKVGTLSTATGPHN PFVDGQGQPVFRGRIQ ANIND
Sbjct: 61 GQKVGTLSTATGPHNSVSIAGKGASVSGGVATVPFVDGQGQPVFRGRIQRANIND

Query: 121 GIDGLAGWRVASSQETLNPVTTFGKSTLPAGTFTATFYVQQYQN 165
GIDG AGWRVASSQETLNPVTTFG+STLPAG FTATFYVQQYQN
Sbjct: 121 GIDGFAGWRVASSQETLNPVTTFGESTLPAGAFTATFYVQQYQN 165

tr Q9X6U1 CS22 adhesin protein [cseA] [Escherichia coli] 1
Q9X6U1_ECOLI a

Score = 92.0 bits (227), Expect = 6e-18
Identities = 55/155 (35%), Positives = 80/155 (51%), Gaps = 4/155

Query: 14 IACGSAHAAGFVGNKXXXXXXXXXXXXXXXXXSANWSQDPGFTGPAVAAGQKVGTLSTI
+ CG+A+AA VG+ +A W+QDP +G +V A QK+GTL+I
Sbjct: 13 MTCGAANAATVVGDVATVQAPVVFSAQNTINATWTQDPSVSGSSVQAMQKLGTINI

Query: 74 PHNXXXXXXXXXXXXXXXXXXXXPFVDGQGQPVFRGRIQGANINDQANTGIDGLA--G
H PF + GQ FRGR A+I +NT I G + G
Sbjct: 73 SHAGVYVSGDGTGVSGGLVTIPFKNAAGQIPFRGR-TNADIGQASNTLIAGHSGPG

Query: 132 SSQETLNPVTTFGKS-TLPAGTFTATFYVQQYQN 165
+ +++ + F K+ +PAGT+TATFY+QQYQ+
Sbjct: 132 DAGNNISLDIKAFQKNDNIPAGTYTATFYIQQYQS 166

tr Q47405 Antigen 8786 [nfaA] [Escherichia coli] 16
Q47405_ECOLI al

Score = 87.4 bits (215), Expect = 1e-16

Identities = 53/155 (34%), Positives = 76/155 (49%), Gaps = 4/155

Query: 14 IACGSAHAAGFVGNKXXXXXXXXXXXXXXXXXSANWSQDPGFTGPAVAAGQKVGTL
+ CG+A+AA VG+ +A W+QD +G +V A QK+GTL+I
Sbjct: 13 MTCGAANAATAVGDVATVRAPLVFSAQNTINATWTQDSSVSGSSVTAMQKLGT
LNI

Query: 74 PHNXXXXXXXXXXXXXXXXXXXXPFVDGQGQPVFRGRIQGANINDQANTGIDGLA--G
H PF + GQ +FRGR A I T I G + G
Sbjct: 73 SHAGVYVSGDDTGESGGLITIPFKNTAGQVLFRRGR-TNAEIGQAMTTPIVGHSGPG

Query: 132 SSQETLNVPVTTT-FGKSTLPAGTFTATFYVQQYQN 165
+Q+ N+ + F + +PAG +TATFY+QQYQ+
Sbjct: 132 GTQDNFNLDIRAFQNANNIPAGEYTATFYIQQYQS 166

tr Q2CNM0 Hypothetical protein precursor [MtheDRAFT_1174]
Q2CNM0_9EURY [Methanosaeta
thermophila PT]

Score = 32.7 bits (73), Expect = 4.3

Identities = 21/65 (32%), Positives = 30/65 (46%), Gaps = 6/65 (9%)

Query: 99 GQGQPVFRG-RIQGANINDQANTGIDGLAGWRVASSQETLNVPVTT-----FGKS
GQG+ G + N N +TG G+ GW + S T + TT + +
Sbjct: 1433 GQKLDVSGYKFNDLNGNGNWDGTGEPGIEGWTIYLSDGTTTISTTTGSDGSYSFT

Query: 153 TFTAT 157
+T T
Sbjct: 1493 KYTIT 1497

tr Q2CNV9 Cna B-type [MtheDRAFT_1089] [Methanosaeta thermophila
Q2CNV9_9EURY PT]

Score = 32.3 bits (72), Expect = 5.6

Identities = 20/62 (32%), Positives = 27/62 (43%), Gaps = 10/62 (16%)

Query: 106 RGRIQGANINDQANTGI-----DGLAGWRVASSQETLNVPVTT-----FGKSTLP
+ I G ND+ N G+ GLAGW + S T T + + L
Sbjct: 1553 KSSIWGTFKFNKNNNGVRDAGEQGLAGWEIRISNSTYTRTAITDSDGWYNFTGLD

Query: 156 AT 157
T
Sbjct: 1613 VT 1614

Database: UniProtKB

Posted date: May 29, 2006 12:59 PM
Number of letters in database: 996,947,944
Number of sequences in database: 3,051,855

Database: /home/local/blastnet/database/EXPASY//UniProtKB.01

Posted date: May 29, 2006 1:00 PM
Number of letters in database: 47,202,236
Number of sequences in database: 133,643

Lambda	K	H
0.315	0.131	0.401

Gapped

Lambda	K	H
0.267	0.0410	0.140

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 263,047,190
Number of Sequences: 3185498
Number of extensions: 8226418
Number of successful extensions: 13863
Number of sequences better than 10.0: 6
Number of HSP's better than 10.0 without gapping: 4
Number of HSP's successfully gapped in prelim test: 2
Number of HSP's that attempted gapping in prelim test: 13853
Number of HSP's gapped (non-prelim): 7
length of query: 165
length of database: 1,044,150,180
effective HSP length: 119
effective length of query: 46
effective length of database: 665,075,918
effective search space: 30593492228
effective search space used: 30593492228
T: 11
A: 40
X1: 16 (7.3 bits)
X2: 38 (14.6 bits)
X3: 64 (24.7 bits)
S1: 41 (21.6 bits)
S2: 70 (31.6 bits)
Wallclock time: 6 seconds

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09457657 PMID: 1361237

Characterisation of monoclonal antibodies against a fimbrial structure of *Salmonella enteritidis* and certain other serogroup D salmonellae and their application as serotyping reagents.

Thorns C J; Sojka M G; McLaren I M; Dibb-Fuller M
Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, Surrey.

Research in veterinary science (ENGLAND) Nov 1992, 53 (3) p300-8,
ISSN 0034-5288--Print Journal Code: 0401300

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A panel of 13 monoclonal antibodies from different hybridomas was produced against a novel salmonella fimbrial antigen expressed predominantly by *Salmonella enteritidis* strains. The specificity of the monoclonal antibodies to this antigen (SEF14) was confirmed by enzyme-linked immunosorbent assay (ELISA) using purified SEF14, immune electron microscopy and, with 11 monoclonal antibodies, the identification of a repeating protein subunit (14,300kDa) on the antigen. Blocking-ELISA with the monoclonal antibodies identified epitopes in at least three, non-overlapping clusters which appeared evenly distributed on SEF14 in immune electron microscopy. The use of the monoclonal antibodies in direct-binding ELISA on a range of salmonella serotypes suggested that the epitopes on SEF14 are highly conserved and were expressed by all the *S enteritidis* strains examined; some strains of *S dublin* and the only strain of *S moscow* available were the only other serotypes that expressed SEF14. A latex agglutination reagent based on a monoclonal antibody was developed and used to test for SEF14 on 280 strains (representing 120 serotypes in 24 serogroups of salmonellae) that had been grown on Sensitest agar for 18 hours at 37 degrees C. All *S enteritidis* strains (64) and most *S dublin* strains (28 of 33) produced SEF14 as did the two strains representing *S blegdam* and *S moscow*. SEF14 was not detected in any other strains of serotypes from serogroup D or from any other serogroup examined.

Descriptors: *Antigens, Bacterial--immunology--IM; *Fimbriae, Bacterial--immunology--IM; *Salmonella--immunology--IM; *Salmonella enteritidis--immunology--IM; *Serotyping--methods--MT; Antibodies, Bacterial; Antibodies, Monoclonal; Enzyme-Linked Immunosorbent Assay; Indicators and Reagents; Latex Fixation Tests; Microscopy, Immunoelectron; Salmonella--classification--CL; Sensitivity and Specificity

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Antigens, Bacterial); 0 (Indicators and Reagents)

Record Date Created: 19930121

Record Date Completed: 19930121

11736659 PMID: 9549856

Characterisation of epitopes of type 1 fimbriae of Salmonella using monoclonal antibodies specific for SEF21 fimbriae of Salmonella enteritidis.

Sojka M G; Carter M A; Thorns C J

Department of Bacteriology, Central Veterinary Laboratory, Surrey, UK.

Veterinary microbiology (NETHERLANDS) Jan 16 1998, 59 (2-3) p157-74,

ISSN 0378-1135--Print Journal Code: 7705469

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Monoclonal antibodies (mAbs) were used to identify and characterise **epitopes** of type 1 (SEF21) fimbriae of *Salmonella enteritidis*. The distribution of the **epitopes** among salmonellas and other enterobacteria was investigated, as well as the influence of growth media and temperatures on their expression. At least four different **epitope** clusters were identified on SEF21 fimbriae of *S. enteritidis*. Two of these clusters were associated with fimbrial haemagglutinins that were either common to all salmonellae tested, or restricted only to *S. enteritidis* and *S. dublin*. The four **epitope** clusters were identified on type 1 fimbriae of most *Salmonella* serotypes, as well as non-haemagglutinating type 2 fimbriae of *S. pullorum* and *S. gallinarum*, and on many other enterobacterial species. The expression of the **epitopes** was affected by growth conditions.

Descriptors: *Antibodies, Monoclonal--immunology--IM; *Antigens, Bacterial--chemistry--CH; * **Epitopes** --analysis--AN; *Fimbriae, Bacterial --immunology--IM; **Salmonella enteritidis* --immunology--IM; Animals; Antibodies, Bacterial--immunology--IM; Antigens, Bacterial--immunology--IM; Binding, Competitive; Enzyme-Linked Immunosorbent Assay; **Epitopes** --immunology--IM; Fimbriae, Bacterial--chemistry--CH; Gene Expression Regulation, Bacterial; Glycerol--metabolism--ME; Guanidine--metabolism--ME; Hemagglutination Inhibition Tests; Hemagglutination Tests; Latex Fixation Tests; Mice; Research Support, Non-U.S. Gov't; *Salmonella enteritidis* --chemistry--CH

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Antigens, Bacterial); 0

07122309 PMID: 2875990

Purification and characterization of fimbriae from Salmonella enteritidis.

Feutrier J; Kay W W; Trust T J

Journal of bacteriology (UNITED STATES) Oct 1986, 168 (1) p221-7,
ISSN 0021-9193--Print Journal Code: 2985120R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A human isolate of *Salmonella enteritidis* which displayed strong pellicle formation during static broth culture and mannose-sensitive hemagglutination produced fimbriae which were morphologically indistinguishable from type 1 fimbriae of members of the family Enterobacteriaceae. Fimbrin was purified to homogeneity, and the apparent molecular weight (M_r , 14,400) was markedly lower than that reported for the type 1 fimbrin of *Salmonella typhimurium* (M_r , 22,100). This fimbrin contained 40% hydrophobic amino acids and lacked cysteine. The sequence of the N-terminal 64 amino acids was determined, and sequence alignment revealed that although the 18 N-terminal residues of the *S. enteritidis* molecule shared considerable homology with *Escherichia coli* and *S. typhimurium* type 1 fimbrins, the *S. enteritidis* fimbrin lacked a 6- to 9-residue terminal sequence present in the other type 1 fimbrins and, after residue 18, shared little homology with the *E. coli* sequence. Antibodies raised to the purified *S. enteritidis* fimbrin bound to surface-exposed conformational epitopes on the native fimbriae and displayed pronounced serospecificity. These antibodies were used in the isolation of a nonfimbriated Tn10 insertion mutant which was unable to hemagglutinate.

Descriptors: *Bacterial Proteins--analysis--AN; *Fimbriae, Bacterial --analysis--AN; *Membrane Glycoproteins; *Membrane Proteins--analysis--AN; *Microfilament Proteins; **Salmonella enteritidis* --ultrastructure--UL; Amino Acid Sequence; Antigens, Bacterial--immunology--IM; Bacterial Proteins--genetics--GE; Bacterial Proteins--immunology--IM; Bacterial Proteins--isolation and purification--IP; Cell Fractionation; DNA Transposable Elements; Hemagglutination; Membrane Proteins--genetics--GE; Membrane Proteins--immunology--IM; Membrane Proteins--isolation and purification--IP; Molecular Weight; Mutation; Research Support, Non-U.S. Gov't; *Salmonella enteritidis* --analysis

TABLE 2. Amino acid composition of fimbrin from *S. enteritidis* strain 27655-3b and type 1 fimbrins of other species

Amino acid	No. of residues/fimbriae subunit			
	<i>S. enteritidis</i>	<i>S. typhimurium</i> ^a	<i>E. coli</i> ^b	<i>K. pneumoniae</i> ^c
Asx	13	22	18/19	27
Thr	17	25	20/19	25
Ser	11	23	9/10	14
Glx	14	19	16/13	17
Pro	8	11	2/2	5
Gly	22	23	21/16	18
Ala	21	34	35/31	30
Val	13	16	14/15	18
Met	0	tr	0/0	2
Ile	5	7	5/4	8
Leu	4	12	14/10	13
Tyr	2	4	2/2	6
Phe	7	9	8/7	6
His	1	3	2/2	2
Lys	4	9	4/3	8
Arg	2	4	2/3	5
Cys	0	0	2/2	4
Trp	1	0	0/0	1
Total no. of residues/mol	145	221	174/158	209
Apparent M_r (10^3)	14.4	22.1	17.1/15.7	21.5
Hydrophobic residues (%) (V,M,I,L,A,F,W, and P)	40	40.5	45.1/43.6	39.7

^a Data from Korhonen et al. (24).^b Data from Salit and Gotschlich (31)/data from Klemm (22). The data from Klemm was calculated based on DNA sequence.^c Data from Fader et al. (11).

not shown). Strain 27655-3b also displayed a weak ability to hemagglutinate erythrocytes of all human blood groups; this HA was inhibited by 0.1 M D-mannose but not by 0.1 M L-fucose or 0.1 M D-galactose. Electron microscopy showed that whereas cells of variant 27655-3a were nonfimbriated, *S. enteritidis* 27655-3b cells were heavily fimbriated, and these fimbriae displayed typical type 1 morphology (Fig. 1A).

Purification of fimbriae. Cells of 27655-3b were surface radiolabeled with ¹²⁵I by using the immobilized lactoperoxidase-glucose oxidase procedure. When whole cell lysates were fractionated by SDS-PAGE with 12.5% acrylamide gels, subsequent autoradiography revealed that a polypeptide of an apparent M_r of 14,400 was strongly radiolabeled (data not shown). SDS-PAGE analysis further indicated that this polypeptide was readily removed from the cells by homogenization and centrifugation. Electron microscopy also showed that this simple procedure removed fimbriae from the cells. For large-scale preparation, fimbriae were collected in 0.15 M ethanolamine buffer and partially purified by a simple protocol involving (NH₄)₂SO₄ precipitation, acetone precipitation, and differential ultracentrifugation. This preparation was contaminated with small amounts of flagella, and final purification was achieved by solubilization of the flagella in 0.2% SDS and collection of the fimbriae by ultracentrifugation. This simple protocol resulted in purification to homogeneity as assessed by Coomassie blue R staining of SDS-PAGE gels (Fig. 2A, lane 2).

Characterization of *S. enteritidis* fimbrin. SDS-PAGE analysis of the purified protein indicated an apparent M_r of 14,400 for the fimbrin subunit, and analysis by urea-peptide gel electrophoresis indicated an apparent M_r of 13,000. The

TABLE 3. N-terminal amino acid sequence of fimbrin of *S. enteritidis* strain 27655-3b and type 1 fimbrins of *E. coli* (22, 40) and *S. typhimurium* (41) aligned for greatest homology

Organism (reference)	Residue ^a
<i>S. enteritidis</i>	1 AGFVGNKA · VVQAAVTIAA QNTTSANWS · QDPGFTGPAY · AAGQKVGTL SITATGPHNS VXIAGXQW
<i>E. coli</i> FimA ^b (22)	2 G-T-HF-GE -N--CAVD - GSVDTQTVQLG - VRTASLAQE G-TSSA--FN IQLNDCDT-Y ASK-AVAF
<i>E. coli</i> 1C ^b (40)	3 -T-HF-GD -N--CAVNT - NSFEQTVN LG - VRSDRLKVE G-KSNP--FT IDLNECESQV SAG--IVF
<i>S. typhimurium</i> (41)	4 ADPTPTSVS G-TIHFEGK L-N--XAVST

^a Amino acid residues are designated by the single-letter nomenclature (20). -, Residues homologous with *S. enteritidis* pilin; ·, deletion introduced into *S. enteritidis* sequence to make a better fit; X, not identified.

^b Sequence deduced from DNA sequence.

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S2	658	RD (unique items)
S3	67	S2 AND (DUBLIN? OR ENTERITIDIS? OR GALLINARIUM? OR PULLORUM?)
S4	26	S3/1999:2006
S5	41	S3 NOT S4
S6	14	S5 AND (EPITOP? OR PARATOPE? OR MAP? OR GEYSEN?)

? t s6/3,kwic/13

Proteins; *Poultry Diseases--diagnosis--DI; *Salmonella Infections, Animal
 --diagnosis--DI; *Salmonella enteritidis--isolation and purification--IP;
 Amino Acid Sequence; Animals; Antibodies, Bacterial--analysis--AN;
 Antibodies, Bacterial--biosynthesis--BI; Antigens, Bacterial--chemistry
 --CH; Antigens, Bacterial--genetics--GE; Antigens, Bacterial--immunology
 --IM; Bacterial Proteins--chemistry--CH; Bacterial Proteins--genetics--GE;
 DNA Primers--chemistry--CH; DNA, Bacterial--chemistry--CH; Egg Yolk
 --microbiology--MI; Electrophoresis, Polyacrylamide Gel--veterinary--VE;
 Enzyme-Linked Immunosorbent Assay--veterinary--VE; Feces--microbiology--MI;
 Fimbriae, Bacterial--chemistry--CH; Fimbriae, Bacterial--immunology--IM;
 Latex Fixation Tests--veterinary--VE; Molecular Sequence Data; Polymerase
 Chain Reaction--veterinary--VE; Poultry Diseases--microbiology--MI;
 Recombinant Proteins--chemistry--CH; Recombinant Proteins--genetics--GE;
 Recombinant Proteins--immunology--IM; Salmonella Food Poisoning
 --prevention and control--PC; Salmonella enteritidis--genetics--GE;
 Salmonella enteritidis--immunology--IM; Sensitivity and Specificity;
 Sequence Analysis, DNA; Specific Pathogen-Free Organisms
 CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antigens, Bacterial);
 0 (Bacterial Proteins); 0 (DNA Primers); 0 (DNA, Bacterial); 0
 (Recombinant Proteins); 0 (sefA protein, Salmonella enteritidis);
 147680-16-8 (Fimbriae Proteins)
 Record Date Created: 19990401
 Record Date Completed: 19990401

9/9/2 (Item 2 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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11921066 PMID: 9748652

Periplasmic and fimbrial SefA from Salmonella enteritidis.

Clouthier S C; Collinson S K; Lippert D; Ausio J; White A P; Kay W W
 Department of Biochemistry and Microbiology, Petch Building, University
 of Victoria, P.O. Box 3055, Victoria, B.C. V8W 3P6, Canada.

Biochimica et biophysica acta (NETHERLANDS) Sep 8 1998, 1387 (1-2)
 p355-68, ISSN 0006-3002--Print Journal Code: 0217513

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Salmonella enteritidis produces thin, filamentous fimbriae composed of
 the fimbrin subunit **SefA**. Although insoluble in most detergents and
 chaotropic agents, these fimbriae were soluble at pH 10.5. Furthermore, in
 sodium dodecyl sulfate, these fibers depolymerized into monomers, dimers
 and other multimers of **SefA**, which precipitated on removal of the
 detergent. In contrast, unassembled periplasmic **SefA** fimbrins purified
 from Escherichia coli expressing cloned **sefA** and **sefB** were readily
 soluble in aqueous solution. Fimbrial and periplasmic **SefA** also differed
 in their reaction with an anti- **SEF14** monoclonal antibody and in their
 surface hydrophobicity, indicating that the two forms had different
 properties. Precise mass measurements of periplasmic and fimbrial **SefA** by
 mass spectroscopy showed that these variations were not due to
 post-translational modifications. Periplasmic **SefA** consisted primarily of
 intact as well as some N-terminally truncated forms. The main 24 amino
 acid, N-terminally truncated form of periplasmic **SefA** was present as a
 12.2 kDa monomer which had a low tendency to dimerize whereas intact
 periplasmic **SefA** was present as a 34.1 kDa homodimer. Intact periplasmic
SefA also formed stable multimers at low concentrations of chemical

cross-linker but multimerization of the truncated form required high concentrations of protein or cross-linker. Thus, **SefA** fimbriins appear to multimerize through their N-termini and undergo a conformational change prior to assembly into fibers. Within these fibers, subunit-subunit contact is maintained through strong hydrophobic interactions.

Descriptors: *Bacterial Proteins--chemistry--CH; *Fimbriae Proteins; *Salmonella enteritidis--chemistry--CH; Cloning, Molecular; Cross-Linking Reagents--metabolism--ME; Periplasm--chemistry--CH; Pili, Sex--chemistry--CH; Protein Conformation; Recombinant Proteins--chemistry--CH; Research Support, Non-U.S. Gov't; Succinimides--metabolism--ME; Ultracentrifugation

CAS Registry No.: 0 (Bacterial Proteins); 0 (Cross-Linking Reagents); 0 (Recombinant Proteins); 0 (Succinimides); 0 (sefA protein, Salmonella enteritidis); 147680-16-8 (Fimbriae Proteins); 82436-77-9 (bis(sulfosuccinimidyl)suberate)

Record Date Created: 19981113

Record Date Completed: 19981113

9/9/3 (Item 3 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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11805270 PMID: 9627964

Thin aggregative fimbriae enhance Salmonella enteritidis biofilm formation.

Austin J W; Sanders G; Kay W W; Collinson S K

Bureau of Microbial Hazards, Health Protection Branch, Health Canada, Ottawa, Ont., Canada.

FEMS microbiology letters (NETHERLANDS) May 15 1998, 162 (2)

p295-301, ISSN 0378-1097--Print Journal Code: 7705721

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; SPACE LIFE SCIENCES

Salmonella enteritidis enteropathogens produce a variety of potentially adherent fimbrial types including **SEF14**, SEF17, SEF18 and SEF21 (type I). In a simplified, pure culture, biofilm generating system the virulent isolate, *S. enteritidis* 3b, readily adhered to Teflon (polytetrafluoroethylene) and stainless steel forming thick cell aggregates. The inability of an isogenic SEF17-deficient mutant to form thick biofilms suggested a role for SEF17 in stabilizing cell-cell interactions during biofilm formation. Epifluorescent detection of SEF17 in biofilms confirmed the association of these fimbriae with aggregated cells but not with adherent mutants unable to produce SEF17. The reduced adherence observed with an isogenic **SEF14**/SEF21-deficient strain implicated the involvement of additional cell surface adherence factors, possibly including SEF21 (type I) fimbriae in the adherence of *S. enteritidis* to stainless steel or Teflon. The role of SEF17 fimbriae in biofilm formation and the contributions of SEF17 to the persistence of *Salmonellae* on surfaces and in food are discussed.

Descriptors: *Biofilms--growth and development--GD; *Fimbriae, Bacterial--physiology--PH; *Salmonella enteritidis--physiology--PH; Bacterial Adhesion--physiology--PH; Blotting, Western; Electrophoresis, Polyacrylamide Gel; Humans; Microscopy, Electron, Scanning; Microscopy, Fluorescence; Research Support, Non-U.S. Gov't; Salmonella enteritidis--growth and development--GD; Salmonella enteritidis--ultrastructure--UL

Record Date Created: 19980709

Record Date Completed: 19980709

9/9/4 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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11706337 PMID: 9511756

Improved allelic exchange vectors and their use to analyze 987P fimbria gene expression.

Edwards R A; Keller L H; Schifferli D M
University of Pennsylvania School of Veterinary Medicine, Philadelphia 19104, USA.

Gene (NETHERLANDS) Jan 30 1998, 207 (2) p149-57, ISSN 0378-1119--
Print Journal Code: 7706761

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A series of vectors has been developed to provide improved positive and negative selection for allelic exchange. Based on homologous regions of DNA ranging in size from less than 200 bp to over 1 kb, we have successfully used these new plasmids to introduce or remove markers in chromosomal or plasmid DNA. Wild type fimbria genes were replaced both in *Salmonella enteritidis* (*sefA* , *agfA* and *fimC*) and *Escherichia coli* (*fasA* and *fasH*). Regulation of 987P fimbriation could be identified after replacement of *fasA* and *fasH* with allelic reporter fusions. The expression of *fasA* but not *fasH* is dependent upon the osmolarity of the growth medium in an HNS-dependent manner, but unlike some other fimbrial systems expression is not dependent on the exogenous iron concentration.

Descriptors: *Adhesins, *Escherichia coli*--genetics--GE; *Antigens, Bacterial--genetics--GE; *Antigens, Surface--genetics--GE; **Escherichia coli*--genetics--GE; *Fimbriae Proteins; *Fimbriae, Bacterial--genetics--GE; *Gene Expression Regulation, Bacterial; *Genetic Vectors; **Salmonella enteritidis*--genetics--GE; Alleles; Bacterial Proteins--genetics--GE; Base Sequence; Chloramphenicol O-Acetyltransferase--genetics--GE; DNA, Bacterial ; DNA-Binding Proteins--genetics--GE; Iron--physiology--PH; Molecular Sequence Data; Osmolar Concentration; Recombinant Fusion Proteins--genetics --GE; Research Support, U.S. Gov't, Non-P.H.S.; beta-Lactamases--genetics --GE

CAS Registry No.: 0 (Adhesins, *Escherichia coli*); 0 (Antigens, Bacterial); 0 (Antigens, Surface); 0 (Bacterial Proteins); 0 (DNA, Bacterial); 0 (DNA-Binding Proteins); 0 (Genetic Vectors); 0 (H-NS protein, bacteria); 0 (Recombinant Fusion Proteins); 0 (antigen 987P, *E. coli*); 147680-16-8 (Fimbriae Proteins); 7439-89-6 (Iron)

Enzyme No.: EC 2.3.1.28 (Chloramphenicol O-Acetyltransferase); EC 3.5.2.6 (beta-Lactamases)

Record Date Created: 19980330

Record Date Completed: 19980330

9/9/5 (Item 5 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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11680866 PMID: 9473037

tRNA(Arg) (*fimU*) and expression of SEF14 and SEF21 in *Salmonella enteritidis*.

Clouthier S C; Collinson S K; White A P; Baner P A; Kay W W
Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada.

Journal of bacteriology (UNITED STATES) Feb 1998, 180 (4) p840-5,
ISSN 0021-9193--Print Journal Code: 2985120R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

A Tn10 insertion affecting **SEF14** fimbrial synthesis in *Salmonella enteritidis* was located 13 bp upstream of a gene designated fimU. The 77-bp DNA sequence of fimU from *S. enteritidis* was identical to that of fimU encoding tRNA(Arg) (UCU) from *Salmonella typhimurium* and 96% identical to that of the *Escherichia coli* argU homolog. Furthermore, the open reading frame adjacent to and overlapping the 3' end of fimU was similar to the prophage DLP12 integrase gene. The fimU-encoded transcript comigrated with total cellular tRNA and was predicted to form a tRNA-like cloverleaf structure containing the arginine anticodon UCU. Thus, fimU encoded a tRNA(Arg) specific for the rare codon AGA. fimU mapped to the SEF21 fim operon located 15 C's from the **sef14** gene cluster. Although fimU was located within the SEF21 fim gene cluster, the fimU Tn10 insertion mutant of *S. enteritidis* was found to be defective in **SEF14** as well as SEF21 (type 1) fimbria production. SEF17 and SEF18 fimbria production was not affected. Complementation of this mutant with plasmid-borne fimU restored normal production of the fimbrins **SefA** and **FimA** as well as their respective fimbriae **SEF14** and SEF21. This is the first description of tRNA simultaneously controlling the production of two distinct fimbriae.

Descriptors: *Antigens, Bacterial; *Bacterial Proteins--genetics--GE; *Fimbriae Proteins; *Fimbriae, Bacterial--genetics--GE; *RNA, Transfer, Arg--genetics--GE; **Salmonella enteritidis*--genetics--GE; Base Sequence; Chromosome Mapping; Comparative Study; DNA Transposable Elements; Fimbriae, Bacterial--classification--CL; Fimbriae, Bacterial--metabolism--ME; Gene Expression Regulation, Bacterial; Genes, Bacterial; Genetic Complementation Test; Molecular Sequence Data; Mutagenesis, Insertional; Research Support, Non-U.S. Gov't; Sequence Homology, Nucleic Acid; Species Specificity; Transcription, Genetic

Molecular Sequence Databank No.: GENBANK/AF013136

CAS Registry No.: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (DNA Transposable Elements); 0 (RNA, Transfer, Arg); 0 (SEF21 protein, *Salmonella enteritidis*); 0 (sefA protein, *Salmonella enteritidis*); 147680-16-8 (Fimbriae Proteins)

Record Date Created: 19980310

Record Date Completed: 19980310

9/9/6 (Item 6 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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11231144 PMID: 9009334

Role of SefA subunit protein of SEF14 fimbriae in the pathogenesis of *Salmonella enterica* serovar Enteritidis.

Ogunniyi A D; Kotlarski I; Morona R; Manning P A

Department of Microbiology and Immunology, The University of Adelaide, South Australia.

Infection and immunity (UNITED STATES) Feb 1997, 65 (2) p708-17,
ISSN 0019-9567--Print Journal Code: 0246127

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxibib

In this study, the role of the **SefA** subunit protein of **SEF14** fimbriae in the pathogenesis of *Salmonella enterica* serovar Enteritidis was investigated. This was accomplished by mutating the **sefA** gene in the chromosome of two strains of *S. enterica* serovar Enteritidis by allelic exchange with a copy that has been inactivated by interruption with a nonpolar kanamycin resistance (**aphA-3**) cassette. The effect of this mutation on the ability of the *S. enterica* serovar Enteritidis strains to colonize the intestinal epithelium and to invade other tissues was assessed in BALB/c mice and in vitro by adherence and invasion of HeLa cells. Our results show that an avirulent *S. enterica* serovar Enteritidis vaccine strain, 11RX (no somatic antigen; flagellum antigen phase 1, g,m; flagellum antigen phase 2, -), colonized better and persisted longer in the Peyer's patches of these mice than did its **SefA**-deficient counterpart. However, no such difference was observed between a highly virulent *S. enterica* serovar Enteritidis strain, 7314 (somatic antigen, O1, O9, O12; flagellum antigen phase 1, g,m; flagellum antigen phase 2 [1,7]), and its **SefA**-deficient isogenic mutant. These findings were correlated with in vitro adherence and invasion of HeLa cells. Furthermore, we could not demonstrate a role for **SefA** in the virulence of *S. enterica* serovar Enteritidis as assessed by 50% lethal dose determinations. The implications of these findings are discussed.

Descriptors: *Bacterial Proteins--physiology--PH; *Fimbriae Proteins; *Fimbriae, Bacterial--physiology--PH; *Salmonella Infections, Animal--microbiology--MI; *Salmonella enteritidis--pathogenicity--PY; Animals; Bacterial Proteins--genetics--GE; Base Sequence; Blotting, Southern; Cell Adhesion; Cloning, Molecular; Cosmids; Genes, Bacterial; HeLa Cells--microbiology--MI; Humans; Intracellular Fluid--microbiology--MI; Mice; Mice, Inbred BALB C; Molecular Sequence Data; Mutagenesis, Insertional; Research Support, Non-U.S. Gov't; Restriction Mapping; Salmonella enteritidis--genetics--GE; Salmonella enteritidis--growth and development--GD

Molecular Sequence Databank No.: GENBANK/X98516

CAS Registry No.: 0 (Bacterial Proteins); 0 (Cosmids); 0 (**sefA** protein, *Salmonella enteritidis*); 147680-16-8 (Fimbriae Proteins)

Record Date Created: 19970221

Record Date Completed: 19970221

9/9/7 (Item 7 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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11099867 PMID: 8921734

Seroreactivity of Salmonella-infected cattle herds against a fimbrial antigen in comparison with lipopolysaccharide antigens.

Hoorfar J; Lind P; Bell M M; Thorns C J

Danish Veterinary Laboratory, Copenhagen, Denmark, UK.

Zentralblatt fur Veterinarmedizin. Reihe B. Journal of veterinary medicine. Series B (GERMANY) Oct 1996, 43 (8) p461-7, ISSN 0514-7166

--Print Journal Code: 0331325

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The IgG seroreaction of *Salmonella*-infected cattle herds against a fimbrial antigen (**SEF14**) was compared with that against

lipopolysaccharide (LPS) antigens. Sera from 23 dairy herds (n = 205) from an island with no occurrence of salmonellosis, four herds (n = 303) with recent outbreaks of *S. dublin* and four herds (n = 168) with recent outbreaks of *S. typhimurium*, were tested in a **SEF14** -ELISA, *S. dublin* LPS (0:1, 9, 12) ELISA and *S. typhimurium* LPS (0:1, 4, 5, 12) ELISA. At a cut-off OD of 0.5, only one of the animals tested from the salmonellosis-free island showed significant seroreaction against the **SEF14** antigen, which was confirmed in a Western-blot analysis. Three out of the four *S. dublin*-infected herds had several seroreactors in the **SEF14** -ELISA, whereas all the four herds were positive in the 0:1, 9, 12-ELISA. All but two samples (both from the same herd) in the four *S. typhimurium*-infected herds, positive in the 0:1, 4, 5, 12-ELISA, had OD values below 0.5 in the **SEF14** -ELISA. The results indicate that cattle can produce detectable specific antibodies against fimbrial antigens which may be used for screening of *S. dublin*-infected herds, particularly in areas with low prevalence of salmonellosis, increasing the predictive value of serology.

Tags: Female

Descriptors: *Antigens, Bacterial--immunology--IM; *Cattle--immunology--IM; *Cattle Diseases--immunology--IM; *Lipopolysaccharides--immunology--IM; *Salmonella Infections, Animal--immunology--IM; *Salmonella typhimurium--immunology--IM; Animals; Antigens, Bacterial--metabolism--ME; Blotting, Western--veterinary--VE; Cattle--microbiology--MI; Cattle Diseases--metabolism--ME; Comparative Study; Electrophoresis, Agar Gel--veterinary--VE; Enzyme-Linked Immunosorbent Assay--veterinary--VE; Research Support, Non-U.S. Gov't; Salmonella Infections, Animal--metabolism--ME

CAS Registry No.: 0 (Antigens, Bacterial); 0 (Lipopolysaccharides)

Record Date Created: 19970123

Record Date Completed: 19970123

9/9/8 (Item 8 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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11041468 PMID: 8865172

Diagnostic potential of sefA DNA probes to Salmonella enteritidis and certain other O-serogroup D1 Salmonella serovars.

Doran J L; Collinson S K; Clouthier S C; Cebula T A; Koch W H; Burian J; Banser P A; Todd E C; Kay W W

Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada.

Molecular and cellular probes (ENGLAND) Aug 1996, 10 (4) p233-46, ISSN 0890-8508--Print Journal Code: 8709751

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Salmonella enteritidis thin fimbriae, **SEF14**, were found to be restricted to *S. dublin* and the predominantly poultry-associated members of the *Salmonella* O-serogroup D1, *S. enteritidis*, *S. berta*, *S. gallinarum* and *S. pullorum*, when tested by Western and ELISA analysis from among 90 *Salmonella* isolates of 42 serovars, as well as from members of several related genera of the Enterobacteriaceae. These five serovars and a single isolate of *S. typhi* (D1) were also detected by hybridization of genomic DNA from 732 *Salmonella* isolates of 117 serogroups to gene probes derived from the *S. enteritidis* **sefA** (fimbriin gene), **sefB** (chaperone) or **sefC** (outer

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Escherichia coli strain SM101 harbors a temperature-sensitive allele (lpxA2) of the gene encoding UDP-Glc-NAC acyltransferase (the first enzyme of the lipid A pathway). SM101 is temperature-sensitive for lipid A biosynthesis and growth. To determine whether or not *E. coli* mutants lacking lipid A can be isolated, we examined temperature-resistant revertants of SM101. All regained the ability to synthesize lipid A. However, some were not true revertants but had acquired mutations in a neighboring gene (orf17), while retaining the original lpxA2 lesion. Cell extracts of such revertants displayed 2-5 fold reductions in the specific activity of (3R)-hydroxymyristoyl-ACP dehydrase. Wild-type cells that overproduced the protein encoded by orf17 overproduced (3R)-hydroxymyristoyl-ACP dehydrase activity as much as 170-fold, suggesting that orf17 is the structural gene for the dehydrase. The proposed function of orf17 is further supported by its sequence similarity to fabA, the structural gene for (3R)-hydroxydecanoyl dehydrase of *E. coli*. We suggest that bypass of the lpxA2 phenotype by mutations in orf17 may be due to an increased (3R)-hydroxymyristoyl-ACP pool. The orf17 gene (which we now designate fabZ) is not regulated by fadR. However, orf17 may be related to **sefA**, a suppressor of certain lesions in the cell division/lipid A biosynthesis gene, envA.

Descriptors: **Escherichia coli*--genetics--GE; *Fimbriae Proteins; *Genes, Bacterial; *Hydro-Lyases--genetics--GE; *Lipid A--biosynthesis--BI; *Mutation; Amino Acid Sequence; Bacterial Proteins--genetics--GE; Base Sequence; *Escherichia coli*--enzymology--EN; Gene Expression Regulation, Bacterial; Gene Expression Regulation, Enzymologic; Heat; Molecular Sequence Data; N-Acetylactosamine Synthase--genetics--GE; N-Acetylactosamine Synthase--metabolism--ME; Oligodeoxyribonucleotides; Repressor Proteins--genetics--GE; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/M19334

CAS Registry No.: 0 (Bacterial Proteins); 0 (FadR protein, Bacteria); 0 (Lipid A); 0 (Oligodeoxyribonucleotides); 0 (Repressor Proteins); 0 (sefA protein, *Salmonella enteritidis*); 147680-16-8 (Fimbriae Proteins)
Enzyme No.: EC 2.4.1.90 (N-Acetylactosamine Synthase); EC 4.2.1. (Hydro-Lyases); EC 4.2.1.60 (3-hydroxydecanoyl-(acyl-carrier-protein) dehydratase)

Gene Symbol: FabZ; fadR; lpxA2; **sefA**

Record Date Created: 19950130

Record Date Completed: 19950130

9/9/15 (Item 15 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10216577 PMID: 7960117

A *Salmonella enteritidis* 11RX pilin induces strong T-lymphocyte responses.

Ogunniyi A D; Manning P A; Kotlarski I

Department of Microbiology and Immunology, University of Adelaide, Australia.

Infection and immunity (UNITED STATES) Dec 1994, 62 (12) p5376-83,

ISSN 0019-9567--Print Journal Code: 0246127

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Our previous work, using proteins fractionated by sodium dodecyl

sulfate-polyacrylamide gel electrophoresis to define antigens of *Salmonella enteritidis* 11RX able to stimulate T cells from *S. enteritidis* 11RX-primed (BALB/c x C57BL/6)F1 mice, had indicated the presence of a major antigenic determinant of 14 to 18 kDa (H.-M. Vordermeier and I. Kotlarski, Immunol. Cell. Biol. 68:299-305, 1990). The 14-kDa size is similar to that of the monomeric units of one of the fimbrial structures, **SEF14**, produced by a human enteropathogen, *S. enteritidis* 27655 (J. Feutrier, W. W. Kay, and T. J. Trust, J. Bacteriol. 168:221-227, 1986). Here we present data which indicate that *S. enteritidis* 11RX also produces this protein and that it is able to elicit delayed-type hypersensitivity reactions in *S. enteritidis* 11RX-primed animals and to stimulate in vitro proliferation of, and cytokine release from, T cells obtained from these animals, implying that this fimbrial protein is likely to be an important immunogen of *S. enteritidis*. The protein was purified to homogeneity and is free from contamination with lipopolysaccharide. Standard immunoblot analysis with unabsorbed *S. enteritidis* 11RX antiserum and antiserum absorbed with *Salmonella typhimurium* C5 and various strains of *Escherichia coli*, as well as a panel of anti-14-kDa-protein monoclonal antibodies, suggests that this fimbrial protein is not the common antigen expressed by a number of organisms belonging to the family Enterobacteriaceae. Immunogold electron microscopy with one of these monoclonal antibodies confirms that the 14-kDa protein and **SEF14** are identical.

Tags: Female; Male

Descriptors: *Antigens, Bacterial--immunology--IM; *Bacterial Proteins--immunology--IM; *Fimbriae Proteins; *Fimbriae, Bacterial--immunology--IM; *Lymphocyte Activation; *Salmonella enteritidis--immunology--IM; Amino Acid Sequence; Animals; Bacterial Proteins--isolation and purification--IP; Cross Reactions; Cytokines--secretion--SE; Fimbriae, Bacterial--ultrastructure--UL; Hypersensitivity, Delayed--immunology--IM; Mice; Mice, Inbred BALB C; Mice, Inbred C57BL; Microscopy, Immunoelectron; Molecular Sequence Data; Research Support, Non-U.S. Gov't; *Salmonella enteritidis*--classification--CL; *Salmonella enteritidis*--ultrastructure--UL; *Salmonella typhimurium*--immunology--IM; T-Lymphocytes--immunology--IM
CAS Registry No.: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Cytokines); 0 (sefA protein, *Salmonella enteritidis*); 147680-16-8 (Fimbriae Proteins)

Record Date Created: 19941229

Record Date Completed: 19941229

9/9/16 (Item 16 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10189625 PMID: 7934897

Unique fimbriae-like structures encoded by sefD of the SEF14 fimbrial gene cluster of *Salmonella enteritidis*.

Clouthier S C; Collinson S K; Kay W W

Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada.

Molecular microbiology (ENGLAND) Jun 1994, 12 (6) p893-901, ISSN 0950-382X--Print Journal Code: 8712028

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The **SEF14** gene cluster of *Salmonella enteritidis* was recently shown to contain three genes, sefABC, encoding a unique fimbrin, and proteins homologous to fimbrial chaperones and outer membrane proteins (ushers),

respectively. A fourth open reading frame, designated *sefD*, was found immediately downstream of *sefABC* and overlapping *sefC*. The translated protein sequence of *sefD* was unique, but the composition was similar to that of other bacterial fimbriae. *SefD* was produced in abundance by wild-type *S. enteritidis* as shown by Western blot analysis using antibodies raised to affinity-purified, recombinant *SefD*. Furthermore, unusually long, thin, fimbriae-like structures were evident on *S. enteritidis* and *Escherichia coli* by immunoelectron microscopy, but in other bacterial species *SefD* was expressed as amorphous material. Therefore, in *S. enteritidis* and *E. coli*, *SefD* is the predominant structural subunit of SEF18. The SEF18 fimbriae-like structures were shown to be serologically distinct from the three known *S. enteritidis* fimbriae SEF14, SEF17 and SEF21. Furthermore, SEF18 was still produced in *sefA* insertion mutants, indicating that SEF14 and SEF18 were structurally distinct. Thus, the SEF14 gene cluster is the first example in the Enterobacteriaceae of a gene cluster that encodes two fimbrin-like proteins, which are assembled into two distinct cell-surface structures, SEF14 and SEF18. DNA hybridization and Western blot analyses showed that *SefD* was widely distributed among the Enterobacteriaceae and was present in *E. coli*, *Shigella*, *Enterobacter*, *Citrobacter*, *Erwinia*, *Hafnia*, *Klebsiella*, *Providencia*, and *Proteus* but not in the non-Enterobacteriaceae Gram-negative bacteria *Pseudomonas* and *Aeromonas*, or in Gram-positive bacteria *Bacillus* or *Staphylococcus*. (ABSTRACT TRUNCATED AT 250 WORDS)

Descriptors: *Bacterial Proteins--genetics--GE; *Fimbriae Proteins; *Fimbriae, Bacterial--genetics--GE; *Multigene Family--genetics--GE; *Salmonella enteritidis--genetics--GE; Bacterial Proteins--biosynthesis--BI; Bacterial Proteins--chemistry--CH; Bacterial Proteins--immunology--IM; Base Sequence; Cell Adhesion Molecules--genetics--GE; Cloning, Molecular; DNA, Bacterial--analysis--AN; Fimbriae, Bacterial--ultrastructure--UL; Genes, Bacterial--genetics--GE; Molecular Sequence Data; Molecular Weight; Open Reading Frames--genetics--GE; Recombinant Fusion Proteins --biosynthesis--BI; Research Support, Non-U.S. Gov't; Salmonella enteritidis--cytology--CY; Sequence Analysis, DNA; Species Specificity

Molecular Sequence Databank No.: GENBANK/U07129

CAS Registry No.: 0 (Bacterial Proteins); 0 (Cell Adhesion Molecules); 0 (DNA, Bacterial); 0 (Recombinant Fusion Proteins); 0 (*SefD* protein, *Salmonella*); 0 (*sefA* protein, *Salmonella enteritidis*); 147680-16-8 (Fimbriae Proteins)

Gene Symbol: *sefD*

Record Date Created: 19941107

Record Date Completed: 19941107

9/9/17 (Item 17 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10019456 PMID: 8155479

Serological diagnosis of *Salmonella* serotype enteritidis infections in poultry by ELISA and other tests.

Barrow P A

Institute for Animal Health, Compton Laboratory, Newbury, Berkshire, England, UK.

International journal of food microbiology (NETHERLANDS) Jan 1994, 21 (1-2) p55-68, ISSN 0168-1605--Print Journal Code: 8412849

Publishing Model Print

Document type: Journal Article; Review

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Serological methods have increasingly been used for the detection of invasive *Salmonella* serotypes including enteritidis in poultry. Different types of ELISA, particularly indirect or double antibody-blocking assays using a variety of antigens such as lipopolysaccharide, flagella and **SEF14** fimbrial antigen are used as part of control programmes in a number of countries. There are many advantages to using such assays for preliminary screening of flocks prior to using bacteriological culture methods. (63 Refs.)

Descriptors: *Antibodies, Bacterial--blood--BL; *Enzyme-Linked Immunosorbent Assay--veterinary--VE; *Poultry Diseases--diagnosis--DI; *Salmonella Infections, Animal--diagnosis--DI; *Salmonella enteritidis--immunology--IM; Agglutination Tests--veterinary--VE; Animals; Antigens, Bacterial--diagnostic use--DU; Immunoglobulin G--blood--BL; Poultry

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antigens, Bacterial); 0 (Immunoglobulin G)

Record Date Created: 19940519

Record Date Completed: 19940519

9/9/18 (Item 18 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10019455 PMID: 8155478

The use of latex particle agglutination to specifically detect *Salmonella enteritidis*.

Thorns C J; McLaren I M; Sojka M G

Department of Bacteriology, Central Veterinary Laboratory, New Haw, Addlestone, Surrey, England, UK.

International journal of food microbiology (NETHERLANDS) Jan 1994; 21 (1-2) p47-53, ISSN 0168-1605--Print Journal Code: 8412849

Publishing Model Print

Document type: Journal Article; Review

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

This paper reviews the development and evaluation of a latex particle agglutination test to specifically identify cultured *Salmonella enteritidis* organisms. The test is based on the use of two monoclonal antibody-coated latex reagents, one of which detects the recently discovered **SEF14** fimbriae expressed predominantly by *S. enteritidis* and *S. dublin* organisms, while the second reagent detects the H'p' antigen of *S. dublin* flagella. In a series of field trials 141 out of 142 strains of *S. enteritidis* from eighteen phage types were correctly identified by the latex test. A further 175 salmonella isolates representing 35 serotypes were tested and only two false-positives (*S. dublin*) in the latex test were recorded. This is the first rapid serotype specific test for *S. enteritidis* to be developed, and highlights the potential advantage of fimbrial antigens as novel diagnostic antigens of the future. (13 Refs.)

Descriptors: *Latex Fixation Tests; *Salmonella Food Poisoning--microbiology--MI; *Salmonella enteritidis--isolation and purification--IP; Animals; Humans; Salmonella Food Poisoning--diagnosis--DI; Salmonella enteritidis--classification--CL; Sensitivity and Specificity; Serotyping

Record Date Created: 19940519

Record Date Completed: 19940519

9/9/19 (Item 19 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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Clouthier S C; Muller K H; Doran J L; Collinson S K; Kay W W
Department of Biochemistry and Microbiology, University of Victoria,
British Columbia, Canada.

Journal of bacteriology (UNITED STATES) May 1993, 175 (9) p2523-33,
ISSN 0021-9193--Print Journal Code: 2985120R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Salmonella enteritidis produces thin, filamentous fimbriae designated **SEF14**. A 3.9-kb region of a 5.3-kb fragment encoding genes responsible for **SEF14** biosynthesis was sequenced and found to contain three genes, *sefABC*. *sefA* encoded a novel fimbrin, the structural subunit of **SEF14** fimbriae. *sefB* and *sefC* encoded proteins homologous to Escherichia coli and Klebsiella pneumoniae fimbrial periplasmic chaperone proteins and fimbrial outer membrane proteins, respectively, and are the first such genes to be characterized from Salmonella spp. in vitro expression directed by the 5.3-kb DNA fragment identified *SefA*, *SefB*, and *SefC* as approximately 14,000-, 28,000-, and 90,000-M(r) proteins, respectively, which correlated with their predicted amino acid sequences. *sefB* and *sefC* were not expressed in the absence of *sefA*. Primer extension analysis of *sefABC* revealed two major transcription start sites located upstream of *sefA*. Transcription of *sefBC* also initiated from the *sefA* promoter region. Secondary-structure analysis of the mRNA transcript for *sefABC* predicted the formation of two stable stem-loop structures in the intercistronic region between *sefA* and *sefB* indicative of differential regulation of *SefA*, *SefB*, and *SefC* translation. E. coli cells carrying the 5.3-kb DNA fragment of S. enteritidis DNA were unable to assemble distinguishable **SEF14** fimbriae; however, immunogold-labelled **SEF14** fimbriae were displayed on E. coli clones containing a 44-kb DNA fragment which encompassed the 5.3-kb region. Therefore, *sefABC* genes make up part of a complex *sef* operon responsible for the expression and assembly of **SEF14** fimbriae.

Descriptors: *Fimbriae Proteins; *Fimbriae, Bacterial; *Genes, Bacterial--genetics--GE; *Membrane Glycoproteins--genetics--GE; *Microfilament Proteins; *Molecular Chaperones; *Proteins--genetics--GE; *Salmonella enteritidis--genetics--GE; Amino Acid Sequence; Bacterial Proteins--biosynthesis--BI; Bacterial Proteins--genetics--GE; Base Sequence; Chaperonins; Membrane Glycoproteins--isolation and purification--IP; Microscopy, Immunoelectron; Molecular Sequence Data; Nucleic Acid Conformation; Operon--genetics--GE; RNA, Messenger--genetics--GE; Research Support, Non-U.S. Gov't; Salmonella enteritidis--ultrastructure--UL; Sequence Analysis, DNA; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/L11008; GENBANK/L11009; GENBANK/L11010

CAS Registry No.: 0 (Bacterial Proteins); 0 (Chaperonins); 0 (Membrane Glycoproteins); 0 (Microfilament Proteins); 0 (Molecular Chaperones); 0 (Proteins); 0 (RNA, Messenger); 0 (fimbrin); 0 (*sefA* protein, Salmonella enteritidis); 0 (*sefB* protein, Salmonella); 0 (*sefC* protein, Salmonella enteritidis); 147680-16-8 (Fimbriae Proteins)

Gene Symbol: *sefA*; *sefB*; *sefC*

Record Date Created: 19930526

Record Date Completed: 19930526

9/9/21 (Item 21 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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09457657 PMID: 1361237

Characterisation of monoclonal antibodies against a fimbrial structure of Salmonella enteritidis and certain other serogroup D salmonellae and their application as serotyping reagents.

Thorns C J; Sojka M G; McLaren I M; Dibb-Fuller M
Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, Surrey.

Research in veterinary science (ENGLAND) Nov 1992, 53 (3) p300-8,
ISSN 0034-5288--Print Journal Code: 0401300

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A panel of 13 monoclonal antibodies from different hybridomas was produced against a novel salmonella fimbrial antigen expressed predominantly by Salmonella enteritidis strains. The specificity of the monoclonal antibodies to this antigen (SEF14) was confirmed by enzyme-linked immunosorbent assay (ELISA) using purified SEF14 , immune electron microscopy and, with 11 monoclonal antibodies, the identification of a repeating protein subunit (14,300kDa) on the antigen. Blocking-ELISA with the monoclonal antibodies identified epitopes in at least three, non-overlapping clusters which appeared evenly distributed on SEF14 in immune electron microscopy. The use of the monoclonal antibodies in direct-binding ELISA on a range of salmonella serotypes suggested that the epitopes on SEF14 are highly conserved and were expressed by all the S enteritidis strains examined; some strains of S dublin and the only strain of S moscow available were the only other serotypes that expressed SEF14 . A latex agglutination reagent based on a monoclonal antibody was developed and used to test for SEF14 on 280 strains (representing 120 serotypes in 24 serogroups of salmonellae) that had been grown on Sensitest agar for 18 hours at 37 degrees C. All S enteritidis strains (64) and most S dublin strains (28 of 33) produced SEF14 as did the two strains representing S blegdam and S moscow. SEF14 was not detected in any other strains of serotypes from serogroup D or from any other serogroup examined.

Descriptors: *Antigens, Bacterial--immunology--IM; *Fimbriae, Bacterial--immunology--IM; *Salmonella--immunology--IM; *Salmonella enteritidis--immunology--IM; *Serotyping--methods--MT; Antibodies, Bacterial; Antibodies, Monoclonal; Enzyme-Linked Immunosorbent Assay; Indicators and Reagents; Latex Fixation Tests; Microscopy, Immunoelectron; Salmonella--classification--CL; Sensitivity and Specificity

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Antigens, Bacterial); 0 (Indicators and Reagents)

Record Date Created: 19930121

Record Date Completed: 19930121

9/9/24 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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0013449181 BIOSIS NO.: 200200042692

Method of testing for the presence of salmonella serotypes expressing

Salmonella enteritidis fimbrial antigen (SEFA) and reagents therefor

AUTHOR: Thorns C J

AUTHOR ADDRESS: Woking, England, UK**UK

JOURNAL: Official Gazette of the United States Patent and Trademark Office
Patents 1185 (4): p2684 April 23, 1996 1996

MEDIUM: print
PATENT NUMBER: US 5510241 PATENT DATE GRANTED: April 23, 1996 19960423
PATENT CLASSIFICATION: 435-7.3 PATENT ASSIGNEE: THE MINISTER OF
AGRICULTURE, FISHERIES AND FOOD IN HER BRITANNIC MAJESTY'S GOVERNMENT OF
THE U.K. OF GT. BRITAIN and N. IRELAND PATENT COUNTRY: USA
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Citation
LANGUAGE: English
DESCRIPTORS:
MAJOR CONCEPTS: Blood and Lymphatics--Transport and Circulation; Methods
and Techniques; Pathology; Systematics and Taxonomy
BIOSYSTEMATIC NAMES: Microorganisms--Microorganisms
ORGANISMS: microorganism (Microorganisms)
COMMON TAXONOMIC TERMS: Microorganisms
MISCELLANEOUS TERMS: BACTERIA; DIAGNOSTIC TESTING; METHODS
CONCEPT CODES:
15001 Blood - General and methods
12504 Pathology - Diagnostic
30000 Bacteriology, general and systematic
01004 Methods - Laboratory methods
BIOSYSTEMATIC CODES:
01000 Microorganisms

9/9/26 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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0009837041 BIOSIS NO.: 199598304874

**Decreased rate of chicken tissue invasion by a *Salmonella enteritidis* sefA
fimbrial mutant**

AUTHOR: Keller Linda H (Reprint); Schifferli Dieter M
AUTHOR ADDRESS: Univ. Penn. Sch. Vet. Med., New Bolton Cent., Kennett
Square, PA 19348, USA**USA
JOURNAL: Abstracts of the General Meeting of the American Society for
Microbiology 95 (0): p219 1995 1995
CONFERENCE/MEETING: 95th General Meeting of the American Society for
Microbiology Washington, D.C., USA May 21-25, 1995; 19950521
ISSN: 1060-2011
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology;
Dental and Oral System--Ingestion and Assimilation; Genetics; Infection
; Membranes--Cell Biology; Physiology
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic
Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Galliformes--
Aves, Vertebrata, Chordata, Animalia
ORGANISMS: *Salmonella enteritidis* (Enterobacteriaceae); Galliformes
(Galliformes)
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Animals;
Birds; Chordates; Nonhuman Vertebrates; Vertebrates
MISCELLANEOUS TERMS: GENE REGULATION; INVASION; MEETING ABSTRACT;
MUTANT STRAIN; PATHOGENICITY; VIRULENCE FACTORS; WILD TYPE STRAIN;
Meeting Abstract
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
10052 Biochemistry methods - Nucleic acids, purines and pyrimidines

10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
 10506 Biophysics - Molecular properties and macromolecules
 10508 Biophysics - Membrane phenomena
 19001 Dental - General and methods
 19006 Dental - Pathology
 22100 Routes of immunization, infection and therapy
 30500 Morphology and cytology of bacteria
 31000 Physiology and biochemistry of bacteria
 31500 Genetics of bacteria and viruses
 36002 Medical and clinical microbiology - Bacteriology
 37003 Public health - Epizootiology
 37058 Public health: disease vectors - Animate

BIOSYSTEMATIC CODES:
 06702 Enterobacteriaceae
 85536 Galliformes

9/9/27 (Item 4 from file: 5)
 DIALOG(R) File 5:Biosis Previews(R)
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0008761054 BIOSIS NO.: 199395063320

Thin, aggregative fimbriae mediate binding of Salmonella enteritidis to fibronectin

AUTHOR: Collinson S Karen; Doig Peter C; Doran James L; Clouthier Sharon; Trust Trevor J; Kay William W (Reprint)
 AUTHOR ADDRESS: Dep. Biochem. Microbiol. Univ. Victoria, Victoria, British Columbia V8W 3P6, Canada**Canada
 JOURNAL: Journal of Bacteriology 175 (1): p12-18 1993
 ISSN: 0021-9193
 DOCUMENT TYPE: Article
 RECORD TYPE: Abstract
 LANGUAGE: English

ABSTRACT: The binding of human fibronectin and Congo red by an autoaggregative Salmonella enteritidis strain was found to be dependent on its ability to produce thin, aggregative fimbriae, named SEF 17 (for Salmonella enteritidis fimbriae with an apparent fimbrin molecular mass of 17 kDa). Two other fimbrial types produced by S. enteritidis, SEF 14 and SEF 21, were not responsible for the aggregative phenotype or for fibronectin binding. SEF 17-negative TnphoA mutants which retained the ability to produce **SEF14** and SEF21 were unable to bind human fibronectin or Congo red and lost the ability to autoaggregate. Only purified SEF 17 but not purified SEF 14 or SEF 21 bound fibronectin in a solid-phase binding assay. Furthermore, only SEF 17 was able to inhibit fibronectin binding to S. enteritidis whole cells in a direct competition enzyme-linked immunosorbent assay. These results indicate that SEF 17 are the fimbriae responsible for binding fibronectin by this enteropathogen.

REGISTRY NUMBERS: 573-58-0: CONGO RED

DESCRIPTORS:

MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology; Infection; Membranes--Cell Biology; Metabolism; Physiology
 BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
 ORGANISMS: Salmonella enteritidis (Enterobacteriaceae); human (Hominidae)
 COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Animals; Chordates; Humans; Mammals; Primates; Vertebrates
 CHEMICALS & BIOCHEMICALS: CONGO RED
 MISCELLANEOUS TERMS: CONGO RED; MOLECULAR WEIGHT; **SEF 14** ; SEF 17

FIMBRIAE; SEF 21; VIRULENCE FACTOR

CONCEPT CODES:

10064 Biochemistry studies - Proteins, peptides and amino acids
10068 Biochemistry studies - Carbohydrates
10506 Biophysics - Molecular properties and macromolecules
10508 Biophysics - Membrane phenomena
13012 Metabolism - Proteins, peptides and amino acids
15002 Blood - Blood and lymph studies
30500 Morphology and cytology of bacteria
31000 Physiology and biochemistry of bacteria
36002 Medical and clinical microbiology - Bacteriology

BIOSYSTEMATIC CODES:

06702 Enterobacteriaceae
86215 Hominidae

9/9/34 (Item 1 from file: 35)

DIALOG(R)File 35:Dissertation Abs Online

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01534469 ORDER NO: AADNN-13703

CHARACTERIZATION OF THE SEF14 FIMBRIAL GENE CLUSTER AND THE ENCODED FIMBRIAE (SALMONELLA ENTERITIDIS)

Author: CLOUTHIER, SHARON CAROL

Degree: PH.D.

Year: 1995

Corporate Source/Institution: UNIVERSITY OF VICTORIA (CANADA) (0244)

Adviser: W. W. KAY

Source: VOLUME 57/10-B OF DISSERTATION ABSTRACTS INTERNATIONAL.

PAGE 6069. 253 PAGES

Descriptors: BIOLOGY, MOLECULAR

Descriptor Codes: 0307

ISBN: 0-612-13703-1

Salmonella enteritidis produces thin, filamentous fimbriae designated

SEF14 . A 7.1 kb fragment encoding genes responsible for **SEF14** biosynthesis was sequenced and found to contain an IS3 element and five genes, sefABCDE. **sefA** encoded the structural subunit of **SEF14** fimbriae. **sefB** and **sefC** encoded proteins homologous to fimbrial chaperones and ushers, respectively. In vitro expression directed by a 5.3 kb fragment identified **SefA** , **SefB**, and **SefC** as approximately 14K, 28K and 90K M_s proteins, respectively, which correlated with their predicted amino acid sequences. E. coli carrying the same 5.3 kb fragment were unable to assemble **SEF14** fimbriae; however, immunogold labelled **SEF14** fimbriae were displayed on E. coli clones containing a 44 kb fragment which encompassed the 5.3 kb region. Therefore, sefABC comprised only part of the **sef14** operon responsible for the expression and assembly of **SEF14** fimbriae.

Further DNA sequence analysis revealed two open reading frames, designated **sefD** and **sefE** immediately downstream of **sefABC**. **sefD** had the same translational polarity whereas **sefE** had the opposite polarity at **sefABC**. In vitro expression of a 10 kp KpnI fragment identified **SefD** and **SefE** as 18K and 30K M_s proteins, respectively, which correlated with their predicted amino acid sequences. **sefE** encoded a protein homologous to AraC family transcriptional regulators, whereas the translated protein sequence of **sefD** was unique. **SefD** was produced in abundance by wild type S. enteritidis. Furthermore, unusually long, thin, fimbriae were evident on S. enteritidis and Escherichia coli by immunoelectron microscopy. Thus, **SefD** was designated the structural subunit of fimbriae which were shown to be

serologically distinct from the three known *S. enteritidis* fimbriae **SEF14**, **SEF17** and **SEF21** and were given the name **SEF18** fimbriae. **SefD** was widely distributed among Enterobacteriaceae. In addition, **sefD**, as well as **sefA** were mapped to the 90 centisome position on the *S. enteritidis* chromosome.

DNA sequence analysis of the region upstream of **sefA**, revealed three open reading frames, **orfABC**, whose genetic organization and sequence was characteristic of IS3 elements. Furthermore, the 289 bp region between the IS3 element and **sefA** contained three putative deoxyadenosine methylase sites and two consensus integration host factor binding sites.

Production of **SEF14** fimbriae was thermoregulated since these fimbriae were not expressed by *S. enteritidis* grown below 30°C.

SEF14 fimbriae are polymers of the protein **SefA**. In SDS polyacrylamide gels, **SefA** isolated from the periplasm of *E. coli* clone separated into two forms that differed by only 1-2 kDa. Solution analysis revealed that the lower molecular weight form (**SefA**_L) was a monomer whereas the higher form (**SefA**_H) was a dimer. The monomer could be cross-linked to form a dimer but only after **SefA**_L shifter 1-2 kDa higher in the gel. Thus, the cross-linker was substituting for something in **SefA**_L that was missing but required for dimerization. Sequence analysis revealed that **SefA**_L lacked the first 24 N-terminal amino acids which accounted for the lower molecular weight and indicated that these 24 amino acids were required for dimerization. The dimer could be the basic building unit of **SEF14** fimbriae. (Abstract shortened by UMI.)

9/9/38 (Item 3 from file: 65)

DIALOG(R)File 65:Inside Conferences

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01299173 INSIDE CONFERENCE ITEM ID: CN012769076

Role of the SEF14 fimbrial antigen in the colonization and persistence of Salmonella enteritidis infection in poultry

Thorns, C. J.; Turcotte, C.; Woodward, M. J.

CONFERENCE: Protection of poultry from foodborne pathogens-Workshop

P: 61-70

CEC, 1995

ISBN: 9282753093

LANGUAGE: English DOCUMENT TYPE: Conference Papers

CONFERENCE EDITOR(S): Nagy, B.; Nurmi, E.; Mulder, R. W. A.

CONFERENCE SPONSOR: COST Action 97 Hungarian Academy of Sciences
Veterinary Medical Research Institute

CONFERENCE LOCATION: Budapest

CONFERENCE DATE: Jun 1995 (199506) (199506)

BRITISH LIBRARY ITEM LOCATION: OP-EC/3855

DESCRIPTORS: poultry; foodborne pathogens; COST; veterinary medical
research

9/9/39 (Item 4 from file: 65)

DIALOG(R)File 65:Inside Conferences

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00022547 INSIDE CONFERENCE ITEM ID: CN000226476

Fimbriae of Salmonella enteritidis: Molecular Analysis of SEF14 and Vaccine Development Potential

Woodward, M. J.; Thorns, C. J.; Turcotte, C.

CONFERENCE: Biology of salmonella-NATO Advanced Research Workshop

NATO ASI SERIES A LIFE SCIENCES, 1993; VOL 245 P: 79-82

New York, Plenum Press, 1993
ISSN: 0258-1213 ISBN: 0306444925
LANGUAGE: English DOCUMENT TYPE: Conference Papers
CONFERENCE EDITOR(S): Cabello, F.
CONFERENCE LOCATION: Portorosa, Italy
CONFERENCE DATE: May 1992 (199205) (199205)

BRITISH LIBRARY ITEM LOCATION: 6033.648690
DESCRIPTORS: salmonella; biology; NATO

9/9/56 (Item 4 from file: 266)

DIALOG(R) File 266:FEDRIP

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00510881

IDENTIFYING NO.: 0023553 AGENCY CODE: AGRIC

REDUCE PREHARVEST SALMONELLA ENTERITIDIS/POULTRY: FIMBRIAL PROTEIN

ASSOCIATE INVESTIGATORS: Nagaraja, K. V.; Halvorson, D. A.; Foster, D.

PERFORMING ORG.: UNIV OF MINNESOTA, VETERINARY PATHOBIOLOGY, ST PAUL, MINNESOTA 55108

TYPE OF AWARD: HATCH |c H

SUMMARY: 1. Clone and express SE fimbrial genes in vitro. 2. Evaluate recombinant fimbrial proteins as subunit vaccines against SE infection in poultry. 3. Express SE fimbrial proteins in a non-pathogenic bacterial carrier for use as oral vaccine to prevent colonization of SE in chickens. The overall project is to reduce colonization of SE in the intestinal tract through the application of a recombinant lactobacillus expressing fimbrial genes of SE as a novel vaccine. The two objectives are 1) to develop a recombinant lactobacillus expressing SE fimbrial genes, 2) to evaluate the recombinant lactobacillus against colonization of SE in chickens. Our plan to achieve this is 1) to clone

and express SE fimbrial genes in vitro, 2) to evaluate recombinant fimbrial proteins as subunit vaccines against SE infection in poultry, 3) to evaluate SE fimbrial proteins in a non-pathogenic bacterial carrier for use as oral vaccine to prevent colonization of SE in chickens. PR epidemiology, serological diagnosis, and pathogenesis. In order to understand the molecular epidemiology of Enteritidis, a repetitive sequence based PCR technique was used. A novel repetitive sequence element, Salmonella Enteritidis repeat element (SERE) was identified in the Enteritidis genome. A SERE based PCR was standardized to fingerprint Enteritidis isolates of diverse origin representing

various phage types. SERE-PCR identified 5 distinct but closely related SERE-PCR patterns among 54 Enteritidis isolates. In addition, 34 Enteritidis isolates of phage type 4 were grouped into 4 distinct closely related SERE-PCR types. Moreover, analysis of 54 strains of other Salmonella serovars including 12 O-serogroup D serovars revealed a unique fingerprint pattern for most of the strains. A latex agglutination test (LAT), an enzyme linked immunosorbent assay (ELISA), and a rapid strip immunoblot assay (RSIA) were developed using a recombinant **SEF14** fimbrial antigen of Enteritidis to specifically identify Enteritidis infected chickens. rSEF14 reacted only with serum

from Enteritidis infected chickens and no positive reactions were observed with serum obtained from birds infected with several Salmonella and other avian pathogens suggesting that rSEF14 antigen is specific for Enteritidis. The rSEF14-ELISA and rSEF14-RSIA identified antibodies in serum from more than 80% of experimentally infected birds during first two weeks of infection and 100% of the birds subsequently. In addition, Enteritidis specific antibodies were also detected in egg yolks of infected hens as early as 6 days post-infection using rSEF14-ELISA and rSEF14 RSIA. The role of **SEF14**, SEF17, and SEF21 fimbrial antigens in Enteritidis

pathogenesis was evaluated. Stable,

single, defined, **sefA** (**SEF14**), **agfA** (SEF17) and **fimA** (SEF21) insertionally inactivated fimbrial gene mutants of Enteritidis were constructed through homologous recombination of DNA material between wild type fimbrial genes and 5,3 truncated fimbrial genes in the suicide plasmid vector pKNOCK-Km. In vitro studies using human enterocyte cell lines and chickens macrophage cell lines did not indicate a major role for these fimbrial antigens in modulating either the invasion of enterocytes or uptake by the macrophages. Similarly, in vivo studies in chickens also revealed no major differences in the ability of these mutant strains to colonize chicken ceca or to invade

liver and spleen. The recombinant **SEF14** , SEF17, and SEF21 antigens were evaluated for protection of chickens against Enteritidis colonization of various tissues. No apparent differences were observed in recovery of Enteritidis from cecum, liver, or spleen of chickens vaccinated with different fimbrial antigens. These results supported our earlier data that the **SEF14** , SEF17, and SEF21 fimbriae may not play a major role in Enteritidis pathogenesis. PB

PROGRESS REPORT SUMMARY: H.Kinde H., L.H. Shivaprasad, B.M. Daft, D.H. Read, A. Ardans, R. Breitmeyer, R. Gireesh, K.V. Nagaraja. 2000.: Pathologic and Bacteriologic findings in 27-week-old commercial laying hen experimentally infected with Salmonella enteritidis Phage Type 4. Avian Dis. 44: 239-248. R. Gireesh, R., S. Munir, M.F. Alexeyev, D.A. Halvorson, C.L. Wells, Nagaraja, K.V. 2000: Pathogenic Role of **SEF14** , SEF17 and SEF21 Fimbria in Salmonella enteritidis Infection of chickens. Applied and Environmental

Microbiology, 66: 1759-1763. R. Gireesh, E. Haverly, D.A. Halvorson, K.E. Ferris, D.C. Lauer and K.V. Nagaraja K.V. 2000: Multidrug-Resistant Salmonella Typhimurium DT104 in Poultry. Journal of Food Protection, 63: 155-161.

DESCRIPTORS: animal diseases; bacterial diseases (animals); poultry; poultry diseases; salmonella enteritidis; vaccines; live vaccines; fimbriae; recombinant proteins; gene cloning; gene expression; subunit vaccines; disease control; lactobacillus; genetic engineering; colonization
? logoff hold

>>>KWIC option is not available in file(s): 399

9/3, KWIC/59 (Item 3 from file: 399)

DIALOG(R) File 399: CA SEARCH(R)

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128164911 CA: 128(14)164911k PATENT

A truncated Sef14 fimbrial protein of Salmonella enteritidis and its use as an antigen in diagnosis and prophylaxis of infection

INVENTOR(AUTHOR): Rajashekara, Gireesh; Nagaraja, Kakambi V.; Kapur, Vivek

LOCATION: USA

ASSIGNEE: Regents of the University of Minnesota; Rajashekara, Gireesh; Nagaraja, Kakambi V.; Kapur, Vivek

PATENT: PCT International ; WO 9803656 A1 DATE: 19980129

APPLICATION: WO 97US12639 (19970718) *US 22191 (19960719)

PAGES: 38 pp. CODEN: PIXXD2 LANGUAGE: English

PATENT CLASSIFICATIONS:

CLASS: C12N-015/31A; C07K-014/255B; G01N-033/50B

DESIGNATED COUNTRIES: AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN; CU; CZ; DE; DK; EE; ES; FI; GB; GE; GH; HU; IL; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MD; MG; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; UA; UG; US; UZ; VN; YU; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM

DESIGNATED REGIONAL: GH; KE; LS; MW; SD; SZ; UG; ZW; AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA;

membrane protein) genes encoding proteins involved in **SEF14** biosynthesis. None of 250 Enterobacteriaceae or 27 other eubacterial isolates tested hybridized to the **sef** probes. The **sefA**, **sefB** and **sefC** genes were amplified from these six Salmonella serovars by PCR using primer pairs designed from **sefA**, **sefB** or **sefC** of *S. enteritidis*. DNA sequencing of **sefA** genes from these five serovars indicated limited sequence variability among **sefA** genes and recognition of individual base pairs which could potentially differentiate certain strains of *S. enteritidis*, *S. dublin* and *S. gallinarum*.

Descriptors: *Bacterial Proteins--genetics--GE; *DNA Probes; *Fimbriae Proteins; *Molecular Chaperones; *Salmonella--genetics--GE; *Salmonella Infections, Animal--diagnosis--DI; *Salmonella enteritidis--genetics--GE; Animals; Bacterial Proteins--analysis--AN; Base Sequence; Chickens; DNA, Bacterial--analysis--AN; Genes, Bacterial--genetics--GE; Intestines --microbiology--MI; Molecular Sequence Data; Multigene Family; Nucleic Acid Hybridization; Pili, Sex--chemistry--CH; Polymerase Chain Reaction--methods --MT; Polymorphism, Restriction Fragment Length; Research Support, Non-U.S. Gov't; Salmonella enteritidis--isolation and purification--IP; Sensitivity and Specificity; Sequence Analysis, DNA

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA Probes); 0 (DNA, Bacterial); 0 (Molecular Chaperones); 0 (**sefA** protein, Salmonella enteritidis); 0 (**sefB** protein, Salmonella); 0 (**sefC** protein, Salmonella enteritidis); 147680-16-8 (Fimbriae Proteins)

Record Date Created: 19961227

Record Date Completed: 19961227

9/9/9 (Item 9 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10920602 PMID: 8733179

Detection of Salmonella enteritidis in eggs by the polymerase chain reaction.

Woodward M J; Kirwan S E

Bacteriology Department, Central Veterinary Laboratory, Addlestone, Surrey.

Veterinary record (ENGLAND) Apr 27 1996, 138 (17) p411-3, ISSN 0042-4900--Print Journal Code: 0031164

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A polymerase chain reaction (PCR) for the specific detection of the gene sequence, **sefA**, encoded by all isolates of *Salmonella enteritidis*, was developed. The PCR could detect as few as four *S. enteritidis* washed bacterial cells but egg contents inhibited the PCR. Eggs spiked with 50 *S. enteritidis* bacterial cells were homogenised, inoculated into buffered peptone water and grown at 37 degrees C for 16 hours, when the PCR was successful. A positive internal control was developed to differentiate between true and false negative PCR results for the detection of *S. enteritidis*. In a limited trial of the egg handling procedures and the PCR, one of 250 chickens' eggs from retail outlets was found to be contaminated with *S. enteritidis*.

Descriptors: *Eggs--microbiology--MI; *Polymerase Chain Reaction; *Salmonella enteritidis--isolation and purification--IP; Animals; Base Sequence; Chickens; Molecular Sequence Data; Research Support, Non-U.S. Gov't

Record Date Created: 19961001
Record Date Completed: 19961001

9/9/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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10914522 PMID: 8737493

Studies into the role of the SEF14 fimbrial antigen in the pathogenesis of Salmonella enteritidis.

Thorns C J; Turcotte C; Gemmell C G; Woodward M J

Central Veterinary Laboratory, Addlestone, Surrey, UK.

Microbial pathogenesis (ENGLAND) Apr 1996, 20 (4) p235-46, ISSN 0882-4010--Print Journal Code: 8606191

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

To investigate the role of the **SEF14** fimbrial antigen in pathogenesis, a single defined **sefA** (**SEF14** -) inactivated mutant of *Salmonella enteritidis* strain LA5 was constructed and tested in a number of biological assay systems. There was no significant difference between the wild-type strain and the isogenic **SEF14** - mutant in their abilities to adhere to and invade HEP-2 epithelial cells or their survival in mouse peritoneal macrophages, whereas the **SEF14** - mutant was ingested more rapidly by isolated human PMN. Both the strains colonized the intestine, invaded and spread systemically in 1 day-old chicks, laying hens and BALB/c mice equally well. A significantly greater number of chicks excreted the wild-type **SEF14** + strain during the first week following infection as compared to those infected with the **SEF14** - mutant. However, similar numbers of chicks excreted the two strains between 2 and 7 weeks after infection. These results indicate that possession of **SEF14** fimbriae alone do not appear to play a significant role in the pathogenesis of *S. enteritidis* although its contribution to virulence may be dependent on the host species infected.

Descriptors: *Antigens, Bacterial--physiology--PH; *Bacterial Proteins--physiology--PH; *Fimbriae Proteins; *Fimbriae, Bacterial--physiology--PH; *Salmonella enteritidis--pathogenicity--PY; Animals; Bacterial Adhesion--genetics--GE; Base Sequence; Chickens; Humans; Liver--microbiology--MI; Macrophages, Peritoneal--microbiology--MI; Mice; Mice, Inbred BALB C; Molecular Sequence Data; Mutagenesis, Insertional; Neutrophils--microbiology--MI; Research Support, Non-U.S. Gov't; Salmonella Infections, Animal--microbiology--MI; Salmonella enteritidis--immunology--IM; Spleen--microbiology--MI; Virulence

CAS Registry No.: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (sefA protein, Salmonella enteritidis); 147680-16-8 (Fimbriae Proteins)

Record Date Created: 19960920

Record Date Completed: 19960920

9/9/11 (Item 11 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

10869397 PMID: 8815085

Development and application of enzyme-linked immunosorbent assay for specific detection of Salmonella enteritidis infections in chickens based

on antibodies to SEF14 fimbrial antigen.

Thorns C J; Bell M M; Sojka M G; Nicholas R A
Central Veterinary Laboratory, New Haw, Addlestone, Surrey, United Kingdom.

Journal of clinical microbiology (UNITED STATES) Apr 1996, 34 (4)
p792-7, ISSN 0095-1137--Print Journal Code: 7505564

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was developed to detect antibodies to the **SEF14** fimbrial antigen (**SEF14** -DAS ELISA) and was evaluated for its use in the specific detection of chicken flocks infected with *Salmonella enteritidis*. The **SEF14** -DAS ELISA successfully discriminated between chickens experimentally infected with *S. enteritidis* and those infected with *S. panama* or *S. typhimurium*, although the **SEF14** responses in adult birds infected with *S. enteritidis* were detectable but low. In contrast, ELISAs used to detect antibodies to lipopolysaccharide (LPS) and flagella were unable to discriminate between the infected groups of chicks and adult birds infected with different *Salmonella* serotypes. LPS and flagellar responses were low and variable in chicks, whereas in adult hens they were found to be consistently strong. When flocks naturally infected with *S. enteritidis* were tested by the **SEF14** -DAS ELISA and ELISAs to detect LPS and flagellar antibodies, it was found that they could all identify the infected flocks, although there was little correlation between individual serum samples. The study shows that the **SEF14** -DAS ELISA may offer advantages over existing assays with comparable sensitivities coupled with higher specificities for the serological detection of *S. enteritidis*-infected chicken flocks.

Tags: Female

Descriptors: *Antibodies, Bacterial--blood--BL; *Chickens; *Enzyme-Linked Immunosorbent Assay--veterinary--VE; *Fimbriae Proteins; *Poultry Diseases --diagnosis--DI; *Salmonella Infections, Animal--diagnosis--DI; *Salmonella enteritidis; Animals; Animals, Newborn; Antibodies, Bacterial--biosynthesis --BI; Antigens, Bacterial; Bacterial Proteins--immunology--IM; Egg Yolk --immunology--IM; Enzyme-Linked Immunosorbent Assay--methods--MT; Humans; Pili, Sex--immunology--IM; Poultry Diseases--immunology--IM; Research Support, Non-U.S. Gov't; Salmonella Food Poisoning--etiology--ET; Salmonella Infections, Animal--immunology--IM; Salmonella enteritidis --immunology--IM; Serologic Tests--methods--MT; Time Factors

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (sefA protein, Salmonella enteritidis); 147680-16-8 (Fimbriae Proteins)

Record Date Created: 19961008

Record Date Completed: 19961008

9/9/12 (Item 12 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10854418 PMID: 8677603

Evaluation of SEF14 fimbrial dot blot and flagellar western blot tests as indicators of *Salmonella enteritidis* infection in chickens.

Cooper G L; Thorns C J

Veterinary Laboratories Agency, New Haw, Addlestone, Surrey.

Veterinary record (ENGLAND) Feb 17 1996, 138 (7) p149-53, ISSN 0042-4900--Print Journal Code: 0031164

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The serological responses to *Salmonella enteritidis* flagella (H: g,m) and its fimbrial antigen **SEF14** were evaluated as indicators of infection in chickens and to confirm serological results obtained by an ELISA using *S enteritidis* lipopolysaccharide (LPS) (O: 9,12) as the detecting antigen. The **SEF14** antigen and flagella were extracted from *S enteritidis* and transferred to nitrocellulose paper for use in Western and dot blot tests. Antisera to 19 salmonella serotypes including *S enteritidis* were raised in rabbits and their cross reactivity to the flagellar and **SEF14** antigens was evaluated. Cross reactivity with the **SEF14** antigen was found in one antiserum, raised against *S blegdam*, and to flagella in eight of 19 antisera raised against various salmonella serotypes, most of which shared the flagellar factors g or m with *S enteritidis*. The intensity of cross reaction to flagella was strongest in *S derby* and *S blegdam* antisera. Antisera raised in chickens against *S typhimurium* and *S panama* did not cross react in either test, and neither did pooled sera from eight-week-old salmonella-free, broiler breeder parent chickens. Field sera from two commercial flocks with no history of salmonella infection were negative when tested by the LPS ELISA. These sera were also negative when tested by the flagellar and **SEF14** blots. *S enteritidis* infection in a commercial laying flock was detected initially when the sera were tested by the LPS ELISA and confirmed in individual and pooled sera by the **SEF14** and flagellar tests. *S enteritidis* PT4 was isolated from this flock post mortem.

Descriptors: *Antibodies, Bacterial--analysis--AN; *Antigens, Bacterial--immunology--IM; *Chickens; *Poultry Diseases--diagnosis--DI; *Salmonella Infections, Animal--diagnosis--DI; *Salmonella enteritidis--immunology--IM; Animals; Antibodies, Bacterial--immunology--IM; Antigens, Bacterial--diagnostic use--DU; Blotting, Western--methods--MT; Blotting, Western--standards--ST; Blotting, Western--veterinary--VE; Cross Reactions; Enzyme-Linked Immunosorbent Assay--veterinary--VE; Evaluation Studies; Fimbriae, Bacterial--immunology--IM; Flagella--immunology--IM; Immune Sera--immunology--IM; Immunoblotting--methods--MT; Immunoblotting--standards--ST; Immunoblotting--veterinary--VE; Poultry Diseases--immunology--IM; Rabbits; Salmonella Infections, Animal--immunology--IM

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antigens, Bacterial); 0 (Immune Sera)

Record Date Created: 19960815

Record Date Completed: 19960815

9/9/13 (Item 13 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10820034 PMID: 8635753

The location of four fimbrin-encoding genes, *agfA*, *fimA*, *sefA* and *sefD*, on the *Salmonella enteritidis* and/or *S. typhimurium* *XbaI*-*BlnI* genomic restriction maps.

Collinson S K; Liu S L; Clouthier S C; Banser P A; Doran J L; Sanderson K E; Kay W W

Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada.

Gene (NETHERLANDS) Feb 22 1996, 169 (1) p75-80, ISSN 0378-1119--
Print Journal Code: 7706761

Contract/Grant No.: RO1AI34829; AI; NIAID

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Four fimbrin-encoding genes, *fimA* (type-1 or SEF21 fimbriae), *agfA* (thin aggregative or SEF17 fimbriae), *sefA* (SEF14 fimbriae and *sefD* (SEF18 fimbriae) from *Salmonella enteritidis* (Se) 27655-3b were located onto the *XbaI*-*BlnI* genomic restriction maps of *Salmonella typhimurium* (St) LT2 and Se strains SSU7998 and 27655-3b. The *XbaI* or *BlnI* genomic fragments carrying these genes were identified by hybridization with labeled oligodeoxyribonucleotides or fimbrin-encoding genes. The fimbrin-encoding genes were not encoded by the virulence plasmids, but were located on chromosomal DNA fragments. The position of each gene on a given *XbaI* fragment was determined by hybridization of a series of *XbaI*-digested genomic DNA samples from previously characterized *Tn10* mutants of Se and St with its respective probe. The *fimA* gene mapped near 13 centisomes (Cs) between *purE884::Tn10* at 12.6 Cs (11.8 min) and *apeE2::Tn10* at 12.8 Cs (12.3 min) beside the first *XbaI* site at 13.0 Cs in St or between *purE884::Tn10* at 12.6 Cs and the *XbaI* site at 13.6 Cs in Se. The *agfA* gene mapped near 26 Cs between *putA::Tn10* and *pyrC691::Tn10* in St, but near 40 Cs between *pncX::Tn10* and the *XbaI* site at 43.3 Cs in Se. This difference in map position was due to the location of *agfA* near one end of the 815-kb chromosomal fragment inverted between Se and St. The *sefA* and *sefD* genes mapped precisely at 97.6 Cs in Se, but were absent from the genome of St LT2. To verify the mapping procedures used herein, *tctC* was also mapped in both *Salmonella* serovars. As expected, *tctC* mapped near 60 Cs in both St and Se, thereby confirming previous studies.

Descriptors: *Antigens, Bacterial; *Fimbriae Proteins; *Fimbriae, Bacterial--genetics--GE; *Genes, Bacterial; *Salmonella enteritidis --genetics--GE; *Salmonella typhimurium--genetics--GE; Bacterial Proteins --genetics--GE; Base Sequence; Cell Adhesion Molecules--genetics--GE; Chromosome Mapping; DNA, Bacterial--genetics--GE; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.; Restriction Mapping
CAS Registry No.: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Cell Adhesion Molecules); 0 (DNA, Bacterial); 0 (SEF21 protein, Salmonella enteritidis); 0 (SefD protein, Salmonella); 147680-16-8 (Fimbriae Proteins)

Record Date Created: 19960709

Record Date Completed: 19960709

9/9/14 (Item 14 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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10268262 PMID: 7806516

An *Escherichia coli* gene (*FabZ*) encoding (3R)-hydroxymyristoyl acyl carrier protein dehydrase. Relation to *fabA* and suppression of mutations in lipid A biosynthesis.

Mohan S; Kelly T M; Eveland S S; Raetz C R; Anderson M S

Department of Biochemistry, Merck Research Laboratories, Rahway, New Jersey 07065.

Journal of biological chemistry (UNITED STATES) Dec 30 1994, 269 (52)

p32896-903, ISSN 0021-9258--Print Journal Code: 2985121R

Publishing Model Print

Document type: Journal Article

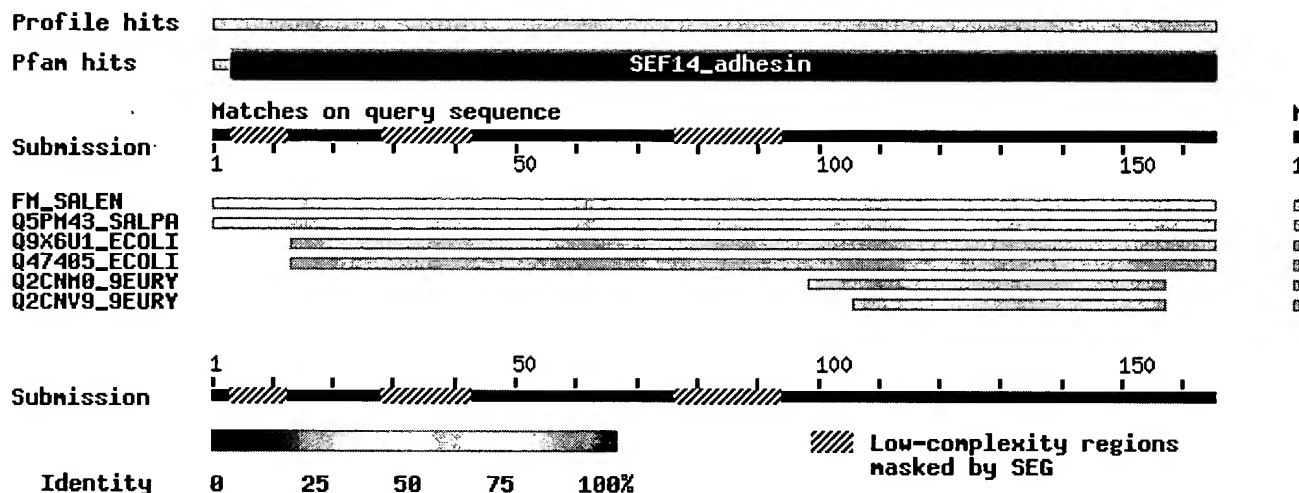
Languages: ENGLISH

Main Citation Owner: NLM

- ☐ tr Q9X6U1 _ECOLI CS22 adhesin protein [cseA] [Escherichia coli]
- ☐ tr Q47405 _ECOLI Antigen 8786 [nfaA] [Escherichia coli]
- ☐ tr Q2CNM0 _9EURY Hypothetical protein precursor [MthedRAFT_1174]
- ☐ tr Q2CNV9 _9EURY Cna B-type [MthedRAFT_1089] [Methanosaeta thermo]

Graphical overview of the alignments

[Click here](#) to resubmit your query after masking regions matching PROSITE profiles or Pfam HMMs
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Alignments

sp P12061 Fimbrial protein precursor [sefA] [Salmonella enteritidis] 1
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Score = 236 bits (603), Expect = 2e-61
 Identities = 122/165 (73%), Positives = 122/165 (73%)

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From: Portner, Ginny
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Ginny Portner
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GN; ML; MR; NE; SN; TD; TG

9/3,KWIC/60 (Item 4 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

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124339735 CA: 124(25)339735c JOURNAL

Studies into the role of the SEE14 fimbrial antigen in the pathogenesis of Salmonella enteritidis

AUTHOR(S): Thorns, C. J.; Turcotte, C.; Gemmell, C. G.; Woodward, M. J.

LOCATION: Central Veterinary Laboratory, Surrey, UK, KT15 3NB

JOURNAL: Microb. Pathog. DATE: 1996 VOLUME: 20 NUMBER: 4 PAGES:

235-246 CODEN: MIPAEV ISSN: 0882-4010 LANGUAGE: English

9/3,KWIC/61 (Item 5 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

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122283875 CA: 122(23)283875n PATENT

Methods and compositions for detection of Salmonella

INVENTOR(AUTHOR): Doran, James L.; Kay, William W.; Collinson, S. Karen; Clouthier, Sharon C.

LOCATION: Can.,

ASSIGNEE: University of Victoria Innovation and Development; King, Joshua

PATENT: PCT International ; WO 9425597 A2 DATE: 941110

APPLICATION: WO 94IB205 (940426) *US 54452 (930426)

PAGES: 95 pp. CODEN: PIXXD2 LANGUAGE: English

PATENT CLASSIFICATIONS:

CLASS: C12N-015/31A; C12Q-001/68B; C12P-021/08B; C07K-014/255B;

G01N-033/56B; C12N-005/12B

DESIGNATED COUNTRIES: AU; BB; BG; BR; BY; CA; CN; CZ; FI; GE; HU; JP; KG; KP; KR; KZ; LK; LV; MD; MG; MN; MW; NO; NZ; PL; RO; RU; SD; SI; SK; TJ; TT; UA; UZ; VN DESIGNATED REGIONAL: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT ; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA; GN; ML; MR; NE; SN; TD; TG

9/3,KWIC/62 (Item 6 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

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122212102 CA: 122(17)212102x PATENT

Cloning of Salmonella genes and vaccines consisting of Salmonella proteins or attenuated Salmonella

INVENTOR(AUTHOR): Kay, William W.; Collinson, S. Karen; Clouthier, Sharon C.; Doran, James L.

LOCATION: Can.,

ASSIGNEE: University of Victoria Innovation and Development; King, Joshua

PATENT: PCT International ; WO 9425598 A2 DATE: 941110

APPLICATION: WO 94IB207 (940426) *US 54452 (930426)

PAGES: 66 pp. CODEN: PIXXD2 LANGUAGE: English

PATENT CLASSIFICATIONS:

CLASS: C12N-015/31A; C07K-014/255B; A61K-039/112B; C12N-001/20B;

C12N-001/20J; C12R-001/42J

DESIGNATED COUNTRIES: AU; BB; BG; BR; BY; CA; CN; CZ; FI; GE; HU; JP; KG; KP; KR; KZ; LK; LV; MD; MG; MN; MW; NO; NZ; PL; RO; RU; SD; SI; SK; TJ; TT; UA; UZ; VN DESIGNATED REGIONAL: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT ; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA; GN; ML; MR; NE; SN; TD;

TG

09744575 PMID: 8371111

Cloning, DNA nucleotide sequence and distribution of the gene encoding the SEF14 fimbrial antigen of Salmonella enteritidis.

Turcotte C; Woodward M J
Molecular Genetics Unit, Central Veterinary Laboratory, Addlestone (Weybridge), Surrey, UK.

Journal of general microbiology (ENGLAND) Jul 1993, 139 (7) p1477-85
, ISSN 0022-1287--Print Journal Code: 0375371

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Monoclonal antibody 69/25, specific for the Salmonella enteritidis fimbrial antigen (**SEF14**), was used to screen a pUC-based S. enteritidis gene library and a positive clone was identified. Subcloning experiments demonstrated that a 584 bp DraI DNA fragment was the minimal chromosomal segment capable of directing **SEF14** antigen expression. Western blotting of Escherichia coli recombinants identified a gene product of M(r) 16000 as a precursor to the M(r) 14300 mature fimbrial subunit protein. The DNA nucleotide sequence of the DraI fragment was determined and was shown to contain a single open reading frame with two potential f-Met start codons and a hydrophobic signal sequence. Downstream of a putative peptidase cleavage site, the deduced amino acid sequence showed considerable homology with the N-terminal amino acid sequence of what was originally described as the type 1 fimbrial subunit of Salmonella enteritidis and later redefined as **SEF14** . The gene encoding **SEF14** , designated as **sefA** , was shown to be limited in distribution to Salmonella blegdam, S. dublin, S. enteritidis, S. gallinarum, S. moscow, S. pullorum, S. rostock, S. seremban and S. typhi, all belonging to Salmonella group D. However, expression of the **SEF14** antigen was limited to S. dublin, S. enteritidis, S. moscow and S. blegdam. The nucleotide sequence of the **sefA** gene shared no homology with the Salmonella fimA gene encoding type 1 fimbriae, and these genes showed distinct patterns of distribution within salmonellae.

Descriptors: *Antigens, Bacterial--genetics--GE; *Bacterial Proteins --genetics--GE; *Fimbriae Proteins; *Genes, Bacterial--genetics--GE; *Salmonella enteritidis--genetics--GE; Amino Acid Sequence; Antibodies, Monoclonal; Base Sequence; Cloning, Molecular; Comparative Study; Conserved Sequence; Gene Expression; Gene Library; Molecular Sequence Data; Regulatory Sequences, Nucleic Acid--genetics--GE; Salmonella typhimurium --genetics--GE; Sequence Alignment; Sequence Analysis, DNA; Sequence Homology, Nucleic Acid; Species Specificity

Molecular Sequence Databank No.: GENBANK/L03833

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antigens, Bacterial) ; 0 (Bacterial Proteins); 0 (fimbriin); 0 (sefA protein, Salmonella enteritidis); 147680-16-8 (Fimbriae Proteins)

Gene Symbol: **sefA**

Record Date Created: 19931008

Record Date Completed: 19931008

9/9/20 (Item 20 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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09619488 PMID: 8097515

Characterization of three fimbrial genes, sefABC, of Salmonella enteritidis.

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• #1 Search **enteritidis epitope fimbriae**

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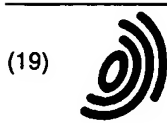
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<input type="checkbox"/>	L16	l9 and l10 and (l13 or l11)	19

END OF SEARCH HISTORY



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Office européen des brevets



(11) **EP 0 551 324 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the grant of the patent:
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(51) Int Cl.⁶: **C12N 15/31, C12N 15/62,
C12Q 1/68, G01N 33/569,
C07H 21/04**

(21) Application number: **91917117.3**

(86) International application number:
PCT/GB91/01691

(22) Date of filing: **01.10.1991**

(87) International publication number:
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(54) **SALMONELLA POLYNUCLEOTIDE SEQUENCE**
POLYNUKLEOTIDSEQUENZ VON SALMONELLA
SEQUENCE DE POLYNUCLEOTIDES DE LA SALMONELLE

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(30) Priority: **01.10.1990 GB 9021338**
17.10.1990 GB 9022570

(43) Date of publication of application:
21.07.1993 Bulletin 1993/29

(73) Proprietor: **THE MINISTER OF AGRICULTURE
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EP-A- 0 383 509 WO-A-89/10967

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September 1988, AMERICAN SOCIETY FOR
MICROBIOLOGY, pages 4216-4222; FEUTRIER,
J. ET AL
- **JOURNAL OF BACTERIOLOGY**, vol. 168, no. 1,
October 1986, AMERICAN SOCIETY FOR
MICROBIOLOGY, pages 221-227; FEUTRIER, J.
ET AL
- **JOURNAL OF GENERAL MICROBIOLOGY**, vol.
136, no. 2, February 1990, COLCHESTER, GB,
pages 265-272; RADFORD, A.J. ET AL

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 0 551 324 B1



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/31, 15/62, G01N 33/569 C12N 1/20, C12P 21/08	A1	(11) International Publication Number: WO 92/06197 (43) International Publication Date: 16 April 1992 (16.04.92)
(21) International Application Number: PCT/GB91/01690 (22) International Filing Date: 1 October 1991 (01.10.91) (30) Priority data: 9021290.3 1 October 1990 (01.10.90) GB 9022570.7 17 October 1990 (17.10.90) GB 9106546.6 27 March 1991 (27.03.91) GB (71) Applicant (for all designated States except US): THE MINISTER FOR AGRICULTURE, FISHERIES AND FOOD IN HER BRITANNIC MAJESTY'S GOVERNMENT OF THE UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND [GB/GB]; Horseferry Road, London SW1P 2AB (GB). (72) Inventor; and (75) Inventor/Applicant (for US only) : THORNS, Christopher, John [GB/GB]; 11 Lincoln Drive, Pyrford, Woking, Surrey GU22 8RL (GB).		(74) Agent: LOCKWOOD, Peter, Brian; Patents 1A, MOD(PE), Room 2121, Empress State Building, Lillie Road, London SW6 1TR (GB). (81) Designated States: AT (European patent), AU, BE (European patent), BG, BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, PL, RO, SE (European patent), SU*, US. Published <i>With international search report.</i>
(54) Title: METHOD OF TESTING FOR SALMONELLA (57) Abstract <p>A method of testing for the presence of Salmonella serotypes <i>S. enteritidis</i> and <i>S. dublin</i> is provided. Novel monoclonal antibodies are used to detect the presence of an epitope specific for these serotypes in cultures which have been grown on selected media which enhance the expression of said epitope in fimbrial sites. Test kits utilising the antigen or its epitopic parts, antibodies and/or the media are further provided.</p>		



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/31, 15/62, G01N 33/569 C12N 1/20, C12P 21/08	A1	(11) International Publication Number: WO 92/06197 (43) International Publication Date: 16 April 1992 (16.04.92)
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(54) Title: METHOD OF TESTING FOR SALMONELLA (57) Abstract <p>A method of testing for the presence of Salmonella serotypes <i>S. enteritidis</i> and <i>S. dublin</i> is provided. Novel monoclonal antibodies are used to detect the presence of an epitope specific for these serotypes in cultures which have been grown on selected media which enhance the expression of said epitope in fimbrial sites. Test kits utilising the antigen or its epitopic parts, antibodies and/or the media are further provided.</p>		

CLAIMS

1. A method of testing for the presence of microorganisms of Salmonella serotypes S. enteritidis or S. dublin comprising exposing an analyte suspected of containing them or their fimbrial antigen (SEFA as described herein) to an antibody raised to said fimbrial antigen or an epitopic part thereof, and then relating the occurrence of antibody-antigen specific binding to the presence of said serotypes.

2. A method of testing for the presence of antibodies to SEFA (as described herein) comprising exposing fimbrial antigen (SEFA as described herein) or an epitopic part thereof to an analyte suspected of containing such antibodies and then relating the occurrence of antibody-antigen specific binding to the presence of said antibodies.

3. A method for the detection of infection by microorganisms of the serotypes S. enteritidis or S. dublin comprising use of a method as claimed in claim 2 to test a suspect biological fluid.

4. A method of determining the identity of a Salmonella serotype as being either S. enteritidis or S. dublin comprising:

(a) exposing an analyte suspected of comprising at least one of said serotypes or their fimbrial antigen (SEFA as described herein) to an antibody raised to said fimbrial antigen, or an epitopic part thereof, and then relating the occurrence of antibody-antigen specific binding to the presence of one of said serotypes:

(b) exposing a further sample of said analyte suspected of comprising at least one of said serotypes to an antibody raised to specifically bind to a first one of said serotypes but not the second and relating the occurrence of antibody-antigen specific binding to the presence of that serotype.

5. A method as claimed in Claim 4 further comprising step (c) of exposing a further sample of said analyte suspected of comprising at least one of said serotypes to an antibody raised to specifically bind to the second one of said serotypes but not to the first and relating the occurrence of antibody-antigen specific binding to the presence of said second serotype.

6. A method of testing for the presence of organisms of *Salmonella* serotypes *S. enteritidis* or *S. dublin* comprising:

(a) seeding a sample of an analyte suspected of containing them into/onto a culture medium selected for its ability to support expression of *Salmonella enteritidis* fimbrial antigen (SEFA);

(b) culturing said seeded material on said culture medium and;

(c) exposing a sample derived from the culture derived from step (b) to an antibody raised to said fimbrial antigen, or a part thereof, and then relating the occurrence of antibody-antigen specific binding to the presence of said serotypes.

7. A method as claimed in Claim 6 wherein the culture medium is selected by screening candidate culture media for the ability to support the expression of SEFA by *S. enteritidis* or a SEFA producing strain of *S. dublin*, wherein the screening comprises identifying antibody-antigen binding between an antibody raised to SEFA or an epitopic part thereof and the *salmonella* cells or fimbriae cultured on said media.

8. A method as claimed in Claim 7 wherein the antibody is one of the monoclonal antibodies MAB 69/25 or MAB 71/3, (deposited as detailed herein).

9. A method as claimed in Claim 7 wherein the antibody is one that has been raised to an antigen which comprises an epitopic part of SEFA and a non-SEFA epitope.

10. A method as claimed in Claim 6 wherein the culture medium is selected from the group comprising Enriched E broth, Heart Infusion broth, peptone water pH 7.2, peptone water pH 6.0, Slanetz broth, desoxycholate citrate agar, McConkey agar, nutrient agar, Salmonella Shigella agar, Sheep blood agar, Xylose Lysine descholate, Medium A (as herein described), Sensitest agar, or Isosensitest agar.
11. A method as claimed in Claim 10 wherein the culture medium consists of Enriched E broth, peptone water pH 7.2, peptone water pH 6.0, Sensitest agar or Isosensitest agar.
12. A method as claimed in Claim 11 wherein the culture medium consists of Sensitest agar or Isosensitest agar.
13. Novel hybridoma cells deposited at the ECACC, Porton Down under Accession numbers 90101101 and 90121902 (as described herein).
14. Novel monoclonal antibodies, MAB 69/25 or MAB 71/3, capable of specifically binding to SEFA (as defined herein), as produced by the hybridoma cells claimed in Claim 13.
15. A test kit for performing the methods as claimed in any one of Claims 1 or in any one of Claims 3 to 12 comprising:
 - (a) cells which are capable of producing antibodies which are capable of specifically binding to SEFA or an epitopic part thereof, and/or (b) said antibodies themselves.
16. A test kit as claimed in Claim 15 comprising
 - (a) hybridoma cells which are capable of producing monoclonal antibodies which are capable of specifically binding to SEFA or an epitopic part thereof, and/or (b) said monoclonal antibodies themselves.

17. A test kit as claimed in Claim 16 wherein the hybridoma cells and/or antibodies are those as claimed in Claim 13 or 14 respectively.
18. A test kit as claimed in Claim 15 or 16 wherein the antibodies are immobilised on a solid carrier.
19. A test kit as claimed in any one of Claims 15 to 18 further comprising an antibody labelling agent.
21. A test kit as claimed in Claim 19 wherein the labelling agent comprises latex particles.
20. A test kit as claimed in any one of Claims 15 to 18 wherein the antibodies are in labelled form.
21. A test kit as claimed in any one of Claims 15 to 21 further comprising the components for preparation of a medium capable of causing or supporting expression of SEFA by S. enteritidis or S. dublin.
22. A test kit as claimed in Claim 21 wherein the components comprise the dry components for preparation of peptone water pH 7.2, peptone water pH 6.0 or a Medium B (as herein described).
23. A test kit as claimed in Claim 22 wherein the Medium B is Sensitest agar or Isosensitest agar.
24. A test kit for use in a method as claimed in Claim 2 comprising Salmonella enteritidis fimbrial antigen (SEFA) or an epitopic part thereof.
25. A test kit as claimed in Claim 24 wherein the SEFA or epitopic part thereof is derived from S. enteritidis or S. dublin microorganisms.
26. A test kit as claimed in Claim 24 where in the SEFA is in the form of detached fimbriae.

27. A test kit as claimed in any one of Claims 24 to 26 wherein the SEFA or epitopic part thereof is immobilised upon a solid substrate.

28. A test kit as claimed in Claim 27 wherein the substrate is a microtitre plate.

29. An isolated polypeptide comprising *Salmonella enteritidis* fimbrial antigen (SEFA as defined herein) or an epitopic part thereof.

30. An isolated polypeptide as claimed in Claim 26 comprising *Salmonella enteritidis* fimbrial antigen (as defined herein).

Fig.1.



Fig.3.

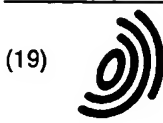


DOCUMENT-IDENTIFIER: WO 9206197 A1

TITLE: METHOD OF TESTING FOR SALMONELLAAbstract Text (1):

CHG DATE=19990617 STATUS=O>A method of testing for the presence of Salmonella serotypes S. enteritidis and S. dublin is provided. Novel monoclonal antibodies are used to detect the presence of an epitope specific for these serotypes in cultures which have been grown on selected media which enhance the expression of said epitope in fimbrial sites. Test kits utilising the antigen or its epitopic parts, antibodies and/or the media are further provided.

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(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
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(51) Int Cl.⁶: **C12N 15/31, C12N 15/62,
C12Q 1/68, G01N 33/569,
C07H 21/04**

(21) Application number: **91917117.3**

(86) International application number:
PCT/GB91/01691

(22) Date of filing: **01.10.1991**

(87) International publication number:
WO 92/06198 (16.04.1992 Gazette 1992/09)

(54) **SALMONELLA POLYNUCLEOTIDE SEQUENCE**
POLYNUKLEOTIDSEQUENZ VON SALMONELLA
SEQUENCE DE POLYNUCLEOTIDES DE LA SALMONELLE

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(74) Representative: **Skelton, Stephen Richard et al**
D/IPR1,
7C/2/A31,
MOD(PE) Abbey Wood,
P.O. Box 702
Bristol BS12 7DU (GB)

(30) Priority: **01.10.1990 GB 9021338**
17.10.1990 GB 9022570

(56) References cited:
EP-A- 0 383 509 **WO-A-89/10967**

(43) Date of publication of application:
21.07.1993 Bulletin 1993/29

(73) Proprietor: **THE MINISTER OF AGRICULTURE**
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- **JOURNAL OF BACTERIOLOGY**, vol. 170, no. 9,
September 1988, **AMERICAN SOCIETY FOR**
MICROBIOLOGY, pages 4216-4222; **FEUTRIER,**
J. ET AL
- **JOURNAL OF BACTERIOLOGY**, vol. 168, no. 1,
October 1986, **AMERICAN SOCIETY FOR**
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ET AL
- **JOURNAL OF GENERAL MICROBIOLOGY**, vol.
136, no. 2, February 1990, **COLCHESTER, GB,**
pages 265-272; **RADFORD, A.J. ET AL**

(72) Inventor: **WOODWARD, Martin, John**
23 Burnsall Close
Hampshire GU14 8NN (GB)

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EP 0 551 324 B1

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 10 TAGTCGTATA TAAATACGAC TCGCTCCAAA TTTTTTTTAT TTCTCGTTTG ATTGTCAACC
 2110 2120 2130 2140 2150 2160

GGACAAATAT ACCAGTGCAG ATTTATTCTGA TAGCGTACCA TTTAGAGGCT TTTCTTTAAA
 15 CCTGTTTATA TGGTCACGTC TAAATAAGCT ATCGCATGGT AAATCTCCGA AAAGAAATTT
 2170 2180 2190 2200 2210 2220

TAAAGATGAA AGTATGATAC CTTTCTCACA GAGAACATAT TATCCAACAA TACGTGGTAT
 20 ATTTCTACTT TCATACTATG GAAAGAGTGT CTCTTGATA ATAGGTTGTT ATGCACCATA
 25 2230 2240 2250 2260 2270 2280

TCGGAAAACC AATGCGACTG TAGAAGTAAG ACAAATGGA TACTTGATAT ATTCTACTTC
 30 ACGCTTTTGG TTACGCTGAC ATCTTCATTG TGTTTTACCT ATGAACTATA TAAGATGAAG
 2290 2300 2310 2320 2330 2340

AGTCCCCCCC GGGCAATTCG AGATAGGTAG AGAACAAATT GCTGATC -3'
 35 TCAGGGGGGG CCCGTTAAGC TCTATCCATC TCTTGTTTAA CGACTAG -5'
 2350 2360 2370 2380

Claims

1. Recombinant DNA encoding

(a) the Salmonella enteritidis fimbrial antigen (SEFA) amino acid sequence:

5 M L I V D F W R F C N M R K S A S A V A V L A L I A C G S A H A A G F
 V G N K A E V Q A A V T I A A Q N T T S A N W S Q D P G F T G P A V A
 A G Q K V G T L S I T A T G P H N S V S I A G K G A S V S G G V A T V
 P F V D G Q G Q P V F R G R I Q G A N I N D Q A N T G I D G L A G W R
 V A S S Q E T L N V P V T T F G K S T L P A G T F T A T F Y V Q Q Y Q
 10 N

(b) an epitopic part thereof, or
 (c) an allelic variant of either

15 the epitopic part and the allelic variant being characterised in that they are capable of specific binding with monoclonal antibody secreted by at least one of the hybridoma cell lines deposited at the ECACC under the accession numbers 90101101 and 90121902.

20 2. Recombinant DNA as claimed claim 1 wherein suitable flanking sequences for control of amino acid sequence expression are provided.

3. Recombinant DNA as claimed in claim 1 or claim 2 comprising the Sequences I and II:

25 Sequence I

5'- G CTCAGAATAC AACATCAGCC AACTGGAGTC AGGAT -3'
 30 3'- C GAGTCTTATG TTGTAGTCGG TTGACCTCAG TCCTA -5'
 230 240 250

35 Sequence II

5'- CCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
 40 3'- GGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
 260 270 280 290 300

45 ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
 TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
 310 320 330 340 350 360

50 TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTT -3'
 55 AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCCTGT CGGACAAAA -5'
 370 380 390 400 410

sequence.

8. Recombinant DNA as claimed in Claim 5 wherein the Sequences V and VI are comprised within a contiguous sequence.
9. Recombinant DNA as claimed in any one of claims 1 to 5 further comprising a sequence encoding for a further amino acid sequence.
10. Recombinant DNA as claimed in claim 9 wherein the further amino acid sequence comprises additional epitopic parts of SEFA, said epitopic parts being characterised in that they are capable of specific binding with monoclonal antibody secreted by at least one of the hybridoma cell lines deposited at the ECACC under the accession numbers 90101101 and 90121902.
11. Recombinant DNA as claimed in claim 9 wherein the further amino acid sequence comprises a non-SEFA epitopic sequence.
12. Recombinant DNA as claimed in claim 11 wherein the non-SEFA epitopic sequence comprises SB10 epitope of Mycobacterium bovis.
13. A novel plasmid comprising recombinant DNA as claimed in any one of claims 1 to 12.
14. A plasmid as claimed in claim 13 comprising a plasmid suitable for transformation of E.coli or yeast into which the recombinant DNA has been inserted.
15. A plasmid as claimed in claim 13 or claim 14 comprising pBR322, pACYC184 or pUC18 into which the recombinant DNA has been inserted.
16. A method for producing a plasmid as claimed in claim 15 comprising the following steps:
 - (a) extracting total genomic DNA from an S. enteritidis or a SEFA expressing S. dublin to produce the recombinant DNA;
 - (b) partially digesting the genomic DNA with SauIIIA restriction endonuclease to provide fragments in the size range 5 to 10 kilobases;
 - (c) ligating the fragments into a plasmid pBR322, pACYC184 or pUC18 and,
 - (d) selecting desired plasmids for their ability to express SEFA, or an epitopic part thereof being characterised in that it is capable of specific binding with monoclonal antibody secreted by at least one of the hybridoma cell lines deposited at the ECACC under the accession numbers 90101101 and 90121902.
17. A method as claimed in claim 16 wherein the desired plasmid comprises a fragment comprising Sequences I and II of claim 3 contiguously and the method further comprises the step of ligating a further DNA sequence into the BamH1 site between the Sequences and in frame with the Sequences.
18. A plasmid obtainable by the method of claim 16 or claim 17.
19. A transformant microorganism comprising a plasmid as claimed in any one of claims 13, 14, 15 or 18.
20. A microorganism as claimed in claim 19 wherein the plasmid host is a yeast or an E.coli.
21. A microorganism as claimed in claim 20 wherein the plasmid host is an E. coli DH5alpha.
22. A polypeptide encoded by the recombinant DNA of claim 11.
23. A test kit for the identification of microorganisms as being of either serotype S. enteritidis or S. dublin comprising a polypeptide or oligopeptide comprising SEFA or an epitopic part thereof as expressed by a transformant as claimed in any one of claims 20 to 22 the epitopic part being characterised in that it is capable of specific binding with monoclonal antibody secreted by at least one of the hybridoma cell lines deposited at the ECACC under the accession numbers 90101101 and 90121902.

□ 1: J Gen Microbiol. 1993 Jul;139(7):1477-85.

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Cloning, DNA nucleotide sequence and distribution of the gene encoding the SEF14 fimbrial antigen of *Salmonella enteritidis*.

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Monoclonal antibody 69/25, specific for the *Salmonella enteritidis* fimbrial antigen (SEF14), was used to screen a pUC-based *S. enteritidis* gene library and a positive clone was identified. Subcloning experiments demonstrated that a 584 bp *Dra*I DNA fragment was the minimal chromosomal segment capable of directing SEF14 antigen expression. Western blotting of *Escherichia coli* recombinants identified a gene product of M(r) 16000 as a precursor to the M(r) 14300 mature fimbrial subunit protein. The DNA nucleotide sequence of the *Dra*I fragment was determined and was shown to contain a single open reading frame with two potential f-Met start codons and a hydrophobic signal sequence. Downstream of a putative peptidase cleavage site, the deduced amino acid sequence showed considerable homology with the N-terminal amino acid sequence of what was originally described as the type 1 fimbrial subunit of *Salmonella enteritidis* and later redefined as SEF14. The gene encoding SEF14, designated as *sefA*, was shown to be limited in distribution to *Salmonella* blegdam, *S. dublin*, *S. enteritidis*, *S. gallinarum*, *S. moscow*, *S. pullorum*, *S. rostock*, *S. seremban* and *S. typhi*, all belonging to *Salmonella* group D. However, expression of the SEF14 antigen was limited to *S. dublin*, *S. enteritidis*, *S. moscow* and *S. blegdam*. The nucleotide sequence of the *sefA* gene shared no homology with the *Salmonella* *fimA* gene encoding type 1 fimbriae, and these genes showed distinct patterns of distribution within salmonellae.

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